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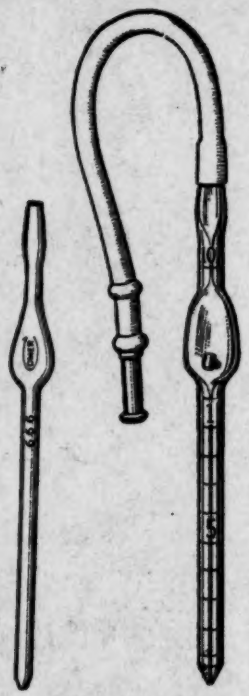

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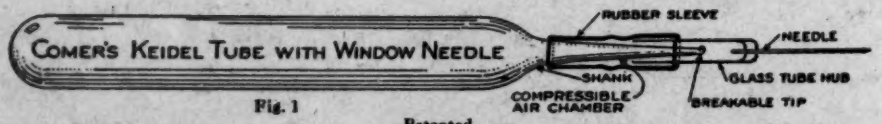

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VOL. XVII ST. LOUIS, MO., FEBRUARY, 1932 NO. 5

CLINICAL AND EXPERIMENTAL

EXPERIMENTAL ADRENAL EXHAUSTION*

BY CHARLES W. EDMUNDS, M.D., AND RALPH G. SMITH, M.D.,
ANN ARBOR, MICHIGAN

NUMEROUS researches have been carried on in the past few years upon the nervous control of the adrenal glands, upon the effect of various poisons upon the output of epinephrine and upon the store of epinephrine which remains in the glands following the action of the poison. The relation of the splanchnic nerves to the glands has also been extensively studied and the importance of these nerves in the control of the output of epinephrine has been repeatedly demonstrated. It has been shown that some of the poisons lead to a certain diminution in the amount of epinephrine in the gland and in fact hunger and cold may have a like effect.

Among the toxins which greatly lessen the epinephrine store, diphtheria toxin occupies a very prominent position. In this connection an important fact was pointed out by Elliott,[1] viz., that the action of this poison is an indirect one and exerted mainly through the central nervous system as its effect is wanting when the splanchnic nerves are cut.

Among the drugs which affect the epinephrine output physostigmine,[2] pilocarpine[3] and strychnine[4] are perhaps the most important. These have been studied by Stewart and Rogoff who have demonstrated that there is a distinct increase in the epinephrine content of the blood following the administrations of these alkaloids. This increase is most marked when physostigmine is given. Certain other drugs are said to have a similar action but the effect is not nearly so pronounced as with those named. One of the less known drugs which apparently possesses this action is a tellurium compound studied by Cow and Dixon[5] who

*From the Laboratory of Pharmacology of the University of Michigan.
Presented before the Association of American Physicians at Atlantic City, May, 1931.

ascribed its effect upon the circulation to the liberation of epinephrine from the adrenals. Section of the splanchnics did not alter the pressor effect of the drug so that it would seem to be due to a direct effect of the compound upon the glands themselves. Repeated injections of the compound tended to produce diminishing results which fact is ascribed to an exhaustion of the glands. A delay, however, before further injections sufficed to produce a normal response again. The rest interval necessary for recovery is not stated but it forms quite an important detail in giving some indication of the rate of epinephrine replacement in the gland. It might be questioned whether the failure of circulatory response to repeated injections is due to lack of available epinephrine or to fatigue of the structure acted upon due to too frequent stimulation. The importance of the point lies in the fact that the conclusion is drawn that during the brief rest interval epinephrine is replaced and that there seemed to be no limit to the amount which the gland can manufacture provided "a little time is given." It will be seen at once that these facts have an important bearing upon the general question of epinephrine replacement, a matter which we have studied in detail, as will be discussed later in this paper. The direct evidence which Cow and Dixon cite in support of this point is found in the epinephrine content of two glands, one removed before, the other after the animal had received six injections of the tellurium compound. Very little difference was found indicating that the gland replenished itself very rapidly. It should be pointed out, however, that a difference in epinephrine content between the two glands would hardly be expected under the conditions described. The amount of active principle which is necessary to raise the blood pressure is so minute that even six stimuli from the tellurium compound would call forth an almost negligible amount of epinephrine. This fact is supported by the results obtained by many investigators who after repeated stimuli of one splanchnic nerve found no lessening of the epinephrine content of the gland as compared with the resting gland.

For instance Stewart, Rogoff and Gibson[6] stimulated the left splanchnic of a cat 52 times in 4 hours. At the end of the experiment the left adrenal yielded 0.14 mg. epinephrine while the right control gland contained 0.10 mg. The situation is much the same when certain drugs are injected which in themselves increase the epinephrine content of the blood but which may or may not affect the store of the active principle. Some lessen the store and others have no such influence.

As an illustration Stewart and Rogoff found that pilocarpine caused a very mild diminution in epinephrine store. In one cat to which 31 mg. had been administered within five hours, both adrenals being the same weight, one gland protected by cutting the splanchnic nerve contained 0.31 mg. of epinephrine while the gland with intact nerve supply had only 0.24 mg. The work of Elliott pointed to a smaller difference even than that quoted above.

Strychnine also was found to increase the epinephrine output even to five or six times the normal amount through a central action and yet examination of the glands on the two sides—one with intact splanchnic and the other with the nerve sectioned—showed no difference in epinephrine store. These cats had been subjected to the action of strychnine for many hours and had apparently been pour-

ing out epinephrine in increased amounts and yet with no diminution of store. The only conclusion to be drawn is that under the influence of the strychnine the animals must have been forming epinephrine in larger amounts to allow for the increased output without depletion of the glands.

Still another drug which has a marked effect upon epinephrine output is physostigmine. It has been shown that this alkaloid will increase the epinephrine content of the blood even to fifteen times the normal amount.[2]

Recently Crowden[7] has shown that the adrenals can be partially depleted by subjecting the animal to external cold. Cats made wet were kept at a temperature of 0° C. for some hours and examination showed that there was a certain degree of depletion in most of the animals. Moreover the depletion was from 20 to 30 per cent greater in the normally innervated gland than it was in the gland with the nerve connection severed. Experiments were carried out also to study the rate of recovery of the glands after such depletion and it was found that while eighteen hours was insufficient, three days seemed to be ample.

Our own experiments were carried out in an attempt to study further the phenomena of adrenal depletion and recovery—what factors might affect them and whether possibly any light might be shed upon the nature of the precursor for epinephrine in the body.

The first part of our study was similar in nature to that carried out by Crowden; viz., to ascertain what agent, if any, could be relied upon to produce epinephrine depletion. We studied first of all physostigmine inasmuch as it had apparently proved to be the most active adrenal stimulant. Our work was done exclusively on dogs and the method of extracting the epinephrine from the glands was essentially that described by Folin, Cannon, and Denis.[8] Metz synthetic epinephrine was employed as a standard, a sufficient amount of N/10 HCl being added to the water containing the alkaloid to bring the latter into solution. A 1:1000 solution thus prepared was further diluted as needed for injection. We found the biologic method of estimating the strength of epinephrine solutions—by means of the blood pressure method on dogs—to be much more satisfactory than the colorimetric method so that we used it exclusively. The details of the manner of carrying out this method of assay are so well known that their repetition here is superfluous. As a control, a total of eleven normal dogs gave an average content of 1.51 mg. of epinephrine for each gram of fresh gland. These figures were a little higher than those usually accepted as being normal for dogs; viz., 0.1 per cent of the moist weight of the glands. Also the individual animals showed a somewhat wide variation, some having as little as 1.04 mg. per gram of gland; others having from 1.75 mg. to 2.00 mg. and one was found with 2.98 mg. per gram. This great variation in epinephrine content made it very difficult to draw conclusions as to the effect of experimental procedures as one animal might normally carry three times as much of the substance as another.

Another source of difficulty encountered at times was the natural tolerance of some of the dogs for physostigmine. While the vast majority of the animals showed marked symptoms from 0.5-1.5 mg. of the alkaloid per kilogram an occasional animal was encountered which was resistant and which showed little effect from such doses. As was to be expected the adrenals of these animals showed

no marked deviation from the normal in epinephrine content. For example, in the first group of "4 or 5 hour" physostigmine dogs one of the group to which was given 0.78 mg. per kg. showed practically no sign of poisoning and its glands yielded a normal amount of epinephrine; viz., 1.16 mg. per gram. This animal was excluded from the final calculations as such an exceptional reaction would complicate the final figures.

Our experiments with physostigmine were accordingly modified from time to time in so far as the dosage and time of administration of the alkaloid were concerned—these being determined by the severity of the toxic symptoms mani-

TABLE I

EXPERIMENTAL RESULTS

Group I. Normal Control Dogs. Average of 11 dogs yielded 1.51 mg. epinephrine per gram of fresh gland.

Group II. "4-5-hour" Physostigmine Dogs.
These animals were injected between 8 and 9 A.M. and the glands removed about 2 P.M. on the same day.

DOSE OF PHYSOSTIGMINE MG. PER KG. BODY WEIGHT	WEIGHT OF ADRENALS	EPINEPHRINE PER GRAM OF GLAND	
0.8	1.08	0.66	
1.5	1.27	0.53	
1.8	1.06	0.84	Average 0.73 mg.
1.0	0.78	0.21	
2.1	1.23	1.40	

Group III. "24-hour" Physostigmine Dogs.
Glands were removed the day following the injection of the physostigmine.

DOSE OF PHYSOSTIGMINE	WEIGHT OF ADRENALS	EPINEPHRINE	
2.99	1.39	0.43*	
1.78	1.11	0.81	
1.77	0.89	0.75	
2.06	0.73	0.27	
2.00	2.04	0.15*	
2.00	0.85	0.88	Average 0.55 mg.

Group IV. "48-hour" Physostigmine dogs.
Glands removed the second day following the injection of physostigmine.

DOSE OF PHYSOSTIGMINE	WEIGHT OF ADRENALS	EPINEPHRINE	
1.74	1.34	0.75	
2.29	1.04	0.44	
1.65	1.21	1.28	
2.00	1.63	2.45	
1.80	1.42	1.41	
4.90	0.9	1.60	
1.90	1.62	1.24	
2.83	1.25	3.20	Average 1.55 mg.

Group V. "72-hour" Physostigmine dogs.
3 days between injection of physostigmine and removal of glands.

DOSE OF PHYSOSTIGMINE	WEIGHT OF ADRENALS	EPINEPHRINE	
1.82	1.3	0.85	
1.58	1.08	1.39	
2.19	1.34	0.87	
2.00	1.14	2.46	
2.08	2.01	3.98	Average 1.91 mg.

fested by the animals. One dog was injected at 11 A.M. and the glands removed at 2 P.M., making only a three-hour interval, and the yield from this animal fell within normal limits; viz., 1.28 mg. per gram of gland. This time then was evidently insufficient to produce any marked change in the gland content as the dose of physostigmine employed (2 mg. per kg. body weight) was ample as shown by numerous subsequent animals. Four more dogs were injected with physostigmine during the early morning hours and in the afternoon. Some four to five hours after the alkaloid had been given the glands were removed and the epinephrine estimated as outlined above. These animals form Group II as given in Table I.

In spite of individual variations the average figures given above yield clear-cut results. Following the subcutaneous administration of an adequate dose of physostigmine the epinephrine content of the adrenals is reduced in four or five hours to about 35 or 40 per cent of the normal. Twenty-four hours later the epinephrine is still further reduced. In certain of the dogs it was as low as from one-fifth to one-tenth of the normal (0.15 mg., 0.27 mg., etc.). The symptoms shown by the dogs with moderately low content were quite striking, very marked weakness and depression. If the epinephrine content is still lower, the depression and weakness is much more marked, and death upon the table usually follows as is indicated by an asterisk in the twenty-four-hour group table.

The forty-eight-hour group of dogs shows that the glands have largely replenished themselves during the second twenty-four-hour period inasmuch as the average content is practically at the normal level. The seventy-two-hour group shows a considerably higher average figure but this is due to the presence in this group of one dog with an abnormally high content, viz., 3.98 mg. If this animal be eliminated from consideration the remaining four give an average within normal limits (1.39 mg.).

The study of the effect of physostigmine upon the adrenals was extended by administering the alkaloid twice daily over a series of days. This method was found to produce a very marked depletion of the medulla of the glands and associated with this poverty of epinephrine were the characteristic weakness and apathy of the animals. Two of the three dogs died on the table and an examination of the adrenals showed extreme epinephrine depletion. One dog weighing 8.6 kg. received an average of 7.5 mg. physostigmine daily, a total of 45 mg. being given. The dog died and the adrenals assayed 0.27 mg. epinephrine per gram of gland. A second dog weighing 6.8 kg. received an average of 8 mg. daily—a total of 98 mg. being given. The animal showed extreme weakness and died on the table, its adrenals yielding 0.45 mg. epinephrine per gram of gland. Such low figures seem to be incompatible with bodily vigor and death usually follows when any extra strain is put upon the animal, even a small dose of urethane being sufficient in several of our dogs to cause a sudden fatal termination. It would seem then to be clearly established that in cases where there has been marked depletion of the adrenal medulla the glands can recover more or less completely in twenty-four hours. It will be remembered that Crowden in his recent paper said that eighteen hours is not sufficient but that three days is ample. From our results it would seem that one day may suffice in some cases.

In addition to the use of physostigmine as a means of causing an increase in the epinephrine content of the blood it has been shown by Stewart and Rogoff[4] that strychnine also has a similar action even when given in doses which are so small that an increase in motor reflexes is not apparent. The action of this alkaloid in increasing the epinephrine output according to these workers is entirely upon the central nervous system as it is absent when the nerves to the gland are cut. A further interesting point concerned the rate of replacement of the epinephrine which had been poured out. It was found that if the nerves to one gland were severed and strychnine injected, the epinephrine content of both glands was essentially the same in all cases. The explanation given was that the epinephrine which had been poured out from the intact gland had been replaced as quickly as it had been excreted so that no depletion was shown. A similar condition had previously been described by Elliott in 1912. Several other workers have studied the action of strychnine upon the adrenals, and all, with one exception, have described an increase in the output of epinephrine and in general the action has been ascribed to an effect upon the central nervous system. Some of these results have been discussed in the paper by Harmon and McFall[9] whose work forms the exceptional finding referred to above. These investigators, employing the denervated heart of cats as a means of estimating epinephrine output, conclude that there is no evidence that strychnine has any action upon the activity of these glands. These findings are not easy to explain as they are at variance with the results reported by so many other workers. The explanation offered by Harmon and McFall is that under the influence of strychnine the animal may struggle due to increased reflex activity and under such conditions an increase in epinephrine may occur but the increase is due to struggling and not directly to the strychnine. This explanation may possibly hold true for certain animals in which strychnine convulsions or struggling have been encountered but would hardly explain the apparent increase in other dogs and cats which may show increased reflex activity but no convulsive movements. Such animals are reported by Edmunds[10] and also by the same writer in collaboration with Lloyd.[11] For example, in the latter paper it was shown that epinephrine in small doses produces characteristic changes in the total white blood cell count and in the relative proportions of the different varieties of the white cells. The same curve of changes follows the injection of small doses of strychnine in animals which are deeply anesthetized but if the adrenals are removed before the strychnine is administered the curve is entirely different. This holds true, as reported in the paper, in animals showing no convulsive movements whatsoever, the effect on the blood being due to the epinephrine excreted under the stimulant action of the strychnine.

If this is correct and epinephrine is excreted in larger amounts under the influence of strychnine, it must also be formed more quickly by the gland, inasmuch as several workers have reported no diminution in the epinephrine content of glands removed after strychnine administration. These findings are hardly to be doubted except that in each case the estimations have been made by the colorimetric method which in our hands at least is not so reliable as the biologic method of assay. It therefore seemed desirable to reexamine the question in view

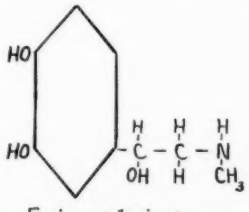
of the findings with physostigmine, and of its bearing upon the general subject of the replacement of this active principle in the gland.

Our experiments which were carried out along the same lines as those when physostigmine effects were studied confirm the reported findings that no lessening of epinephrine content is present even when sufficient strychnine is given to produce muscular twitching. The results obtained on one dog may be cited.

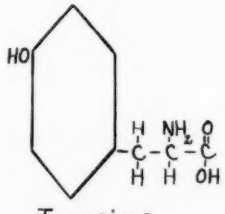
Dog weight, 11 kg. Strychnine sulphate injected subcutaneously as follows: 9:30 A.M. 1 mg.; 11:15 A.M., 0.5 mg.; 12 noon 0.5 mg.; 1 P.M., 0.5 mg. At 1:30 dog showed muscular twitching and some spasticity of legs. 1:45 P.M. dog anesthetized, adrenals removed. Epinephrine content assayed by biologic means and found to be 1.59 mg. per gram of gland. Thus the amount was normal in this animal which showed definite strychnine symptoms.

We tried to produce adrenal exhaustion with strychnine as we had with physostigmine by giving the strychnine over a number of days but with no positive results as the following figures show. Dogs given 0.5 mg. strychnine sulphate subcutaneously twice daily for three days and showing slightly increased reflexes had glands which in one case yielded 1.38 mg. epinephrine per gram of gland and in the other case 2.14 mg. per gram of gland. There was evidently no exhaustion of the glands and whatever epinephrine had been excreted under strychnine stimulation must have been replaced as quickly as it had been poured out. This finding is by no means surprising as it has been repeatedly shown as mentioned above that following numerous stimulations of the splanchnic nerve no lessening in epinephrine content of the glands occurs. There must be a fundamental difference, however, between physostigmine and strychnine in so far as this action is concerned as both increase the outpouring of epinephrine into the blood and both produce this effect by a central action, but while the gland is depleted by the physostigmine no such result follows the use of strychnine or indeed direct stimulation of the splanchnic nerves.

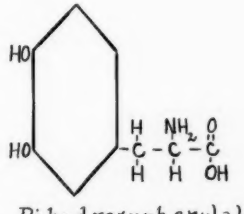
In connection with the synthesis of epinephrine by the animal body there has always been a question as to what is the precursor of the active principle. The two substances which seemed to be the most likely are tyrosine and dihydroxyphenylalanine. These two chemical bodies are quite closely related to epinephrine as can be seen by the following formulae.



Epinephrine



Tyrosine



Dihydroxyphenylalanine

The theory that epinephrine is formed from tyrosine is emphasized by Halle[12] who points out that the four changes in the tyrosine molecule which would be necessary are all perfectly compatible with body metabolism. He further substantiates his views by adding a gram of tyrosine to minced adrenals and incubating the mixture for 7 days. He finds from 14 to 33 per cent more

epinephrine in such a mixture than in one to which no tyrosine has been added. On the other hand Bloch[13] believes that dioxyphenylalanine is a precursor of epinephrine and that in Addison's disease the adrenals are unable to utilize the compound and it therefore is responsible for the pigment formation characteristic of this disease.

In an effort to shed some light upon this question we studied the effect of administering these two compounds to dogs in which the adrenals had been subjected to depletion by physostigmine, as described above. The form of normal recovery curve having been well established, any favorable influence which might be exerted by either of the compounds would show itself probably by a more complete or more rapid recovery than is gained without their use. The experiments were carried out as follows. The dogs were injected with the necessary dose of physostigmine as in the earlier series, and at the end of twenty-four hours when the epinephrine content would be at its lowest level either tyrosine or dihydroxyphenylalanine was administered. In some animals twenty-four hours were allowed following the injection of the suspected precursor before the glands were examined, while in other dogs a second injection was given the next day, so that forty-eight hours were allowed for recovery. The tyrosine solution or suspension caused considerable irritation when it was given subcutaneously so we changed the procedure and gave it intraperitoneally. One-gram doses were stirred for some time in water kept just above body temperature, some NaOH having been added to the mixture. The dihydroxyphenylalanine was prepared in the same way in doses of 200 mg. The results of the combined physostigmine and tyrosine experiments are as follows. It is unnecessary to give all the detailed figures of the thirteen dogs injected with the tyrosine so we will give merely the final assay figures of these animals and the average content in milligrams per gram of gland: 1.31, 1.10, 0.91, 0.83, 0.77, 1.13, 0.51, 0.77, 1.50, 0.66, 2.07, 1.30, 2.25; an average of 1.16 mg. as compared with an average of 1.55 mg. for the dogs which had had no tyrosine. The series of dogs (12) receiving tyrosine on two days yielded results as follows: 1.31, 1.16, 0.84, 0.89, 0.60, 1.81, 0.70, 0.55, 0.62, 0.94, 2.10 mg., an average of 1.04 mg. as compared with 1.91 mg. for the dogs without tyrosine. It is very clear that under the conditions of the experiments tyrosine exerted no favorable influence upon epinephrine replacement which would be expected if it were a precursor of the alkaloid. A similar conclusion was reached by what we may term clinical observation. The dogs given tyrosine were certainly not improved by the drug and in many instances their general condition was worse. A similar study was made with dihydroxyphenylalanine with results which were essentially the same. The glands of three dogs receiving the injections yielded twenty-four hours later epinephrine as follows: 1.50, 2.05, 1.68 mg., an average of 1.74 mg. as compared with the control of 1.55 mg. It is true this figure is higher, but it can hardly be said to be outside the normal limits. An animal given the compound on two days yielded 1.34 mg. of epinephrine as compared with the control figure of 1.91 mg. Dihydroxyphenylalanine therefore does not seem to be beneficial although viewed from other standpoints the negative evidence is not quite so conclusive as it is with tyrosine. For instance we treated dogs with daily injections of dihydroxyphenylalanine for about

two weeks with the following results. One dog weighing 7.7 kg. received 2.7 G. in divided doses between June 5 and 18 and its glands assayed 1.98 mg. per gram of gland. A second dog weighing 10.2 kg. received 1.1 G. between June 20 and July 21 and its glands yielded 1.31 mg., an average for the two of 1.64 mg. which is above the normal average.

Finally as we pointed out earlier there are in all the series of dogs certain of the animals which yield exceptionally high figures, up to three or four times the average value, and such figures with only a few animals in the series profoundly modify the course of the curve. We have accordingly in drawing a graph omitted these exceptional animals from consideration fully realizing that from the statistical point of view such an arbitrary limitation would not be permissible. The justification for such action is seen, of course, when a figure of

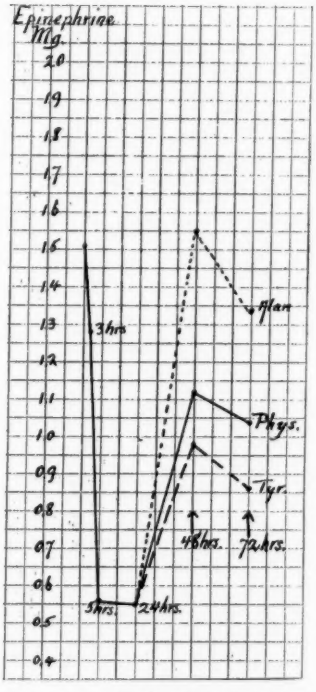


Fig. 1.—Curve of epinephrine content of adrenals of dogs at time periods marked on curves. Solid line shows epinephrine when physostigmine is used alone. Interrupted line (Tyr) when tyrosine is also given as described in the paper, and dotted line (Alan) when dihydroxyphenylalanine is given with the physostigmine.

3.98 mg. is found in a series the other members of which average about 1 mg. Again a dog with 3.20 mg. epinephrine is found in a group averaging about 1.4 mg. Drawing such a curve (Fig. 1) we find that the dihydroxyphenylalanine figures are distinctly above the normal controls in every instance and the findings agree fairly well with the dihydroxyphenylalanine figures for continuous administration. In each instance the seventy-two-hour value is less than the forty-eight-hour figure. Why this should be we cannot suggest, but the parallel findings in the curve would seem to offer some justification for the liberty we have taken in dropping the exceptional figures when the curve is constructed. At the same time these are to be found in the tables given.

SUMMARY

The intramuscular injection of physostigmine in dogs is followed by a diminution in epinephrine content of the adrenal glands at the end of five hours to about one third of the normal, and at the end of thirty hours the depletion is even greater. Such animals exhibit marked symptoms of weakness and apathy. The glands gradually replace the active principle, so that at the end of another twenty-four hours the amount in the glands approaches the normal.

If physostigmine administration is continued over a period of two or more days, the glands are depleted to such an extent that they contain only about an eighth of the normal amount. Such animals are very weak and they may die. Studies were also made upon animals with such depleted adrenals to see whether evidence could be secured upon the existence of a possible precursor substance for the epinephrine. For this purpose tyrosine and also dihydroxyphenylalanine were administered, and the adrenals were then examined at varying intervals to see whether the course of the curve as described above was changed. Tyrosine apparently had no effect, but the dihydroxyphenylalanine seemed to exert a favorable influence; but a positive statement could not be made on account of the wide variations between different animals.

REFERENCES

1. Elliott, T. R.: A Study of the Pathology of the Adrenal Glands in Various Diseases, Quart. J. Med. **8**: 47, 1914.
2. Stewart, G. N., and Rogoff, J. M.: The Action of Drugs upon the Output of Epinephrine from the Adrenals. VII. Physostigmine. J. Pharmacol. & Exper. Therap. **17**: 227, 1921.
3. Stewart, G. N., and Rogoff, J. M.: The Action of Drugs upon the Output of Epinephrine from the Adrenals, VI. Atropine; Pilocarpine; J. Pharmacol. & Exper. Therap. **16**: 71, 1920.
4. Stewart, G. N., and Rogoff, J. M.: The Action of Drugs upon the Output of Epinephrine from the Adrenals. I. Strychnine, J. Pharmacol. & Exper. Therap. **13**: 95, 1919.
5. Cow, D. V., and Dixon, W. E.: The Action of Dimethyltellurium Dihaloids, J. Physiol. **56**: 45, 1922.
6. Stewart, G. N., Rogoff, J. M., and Gibson, F. S.: The Liberation of Epinephrine from the Adrenal Glands by Stimulation of the Splanchnic Nerves and by Massage, J. Pharmacol. & Exper. Therap. **8**: 205, 1916.
7. Crowden, G. P.: The Replacement of Depleted Adrenaline in the Suprarenals, J. Physiol. **68**: 313, 1929.
8. Folin, O., Cannon, W. B., and Denis, W.: A New Colorimetric Method for the Determination of Epinephrine, J. Biol. Chem. **13**: 477, 1913.
9. Harmon, P. M., and McFall, C. M.: The Action of Strychnine upon the Denervated Heart and upon the Secretion of Adrenin, J. Pharmacol. and Exper. Therap. **37**: 131, 1929.
10. Edmunds, C. W.: The Importance of the Adrenal Glands in the Action of Pilocarpine, Physostigmine and Strychnine, J. Pharmacol. & Exper. Therap. **20**: 405, 1923.
11. Edmunds, C. W., and Lloyd, P. C.: The Importance of the Adrenal Glands in the Action of Certain Alkaloids. II. Strychnine on the Blood Picture, J. Lab. & Clin. Med. **8**: 563, 1923.
12. Halle, W. L.: Über die Bildung der Adrenalins in Organismus, Beitr. z. Chem. Physiol. u. Path. **8**: 276, 1916.
13. Bloch, B.: Chemische Untersuchungen über das spezifische pigmentbildende Ferment der Haut, die Dopaoxydase, Ztschr. f. physiol. Chem. **98**: 226, 1917.

POLIOMYELITIS*

By Robert W. Meals, M.D., and Albert G. Bower, M.D., Los Angeles, Calif.

With poliomyelitis again making its appearance in Southern California, a review of the 1930 epidemic in this locality is apropos at this time. Particularly is this true in view of the difference in clinical findings and treatment of our cases from those reported elsewhere. The following remarks are based on observations from 350 cases.

The known etiologic facts concerning polio have been reviewed extensively in the literature the past year and only such points as are pertinent to this discussion will be mentioned. The spread by direct contact or "healthy carriers" was exemplified. One of the Los Angeles City Health Department inspectors who speaks Mexican fluently, made a trip through Sonora and Baja California, just preceding the outbreak of the epidemic in this state. He found large numbers of funerals being conducted in every Mexican town and hamlet and was assured that children were dying from the disease "which leaves them paralyzed if they get well." Soon after this the disease manifested itself in Imperial, San Diego, and Riverside counties, from whence it was traced directly within a few days into Los Angeles County. It is also interesting to note that from here it spread directly north into California as well as to the states east of us along the mainly traveled United States highways.

A normally high immunity is accepted,[1] and whether natural or acquired by previous, mild, unrecognized infection, the use, in a few instances, of pooled, nonspecific adult sera gave results comparable to those obtained with convalescent serum. A shortage of the latter made this procedure necessary early in the epidemic.

The invasion symptoms most frequently encountered were those common to most toxemias, particularly those of an acute infectious nature. Frontal headaches, pain and stiffness of the neck and back, constipation with nausea or vomiting, and some degree of fever were the most constant. Little children, among whom headache of any type is a rare complaint, and who usually pay little attention to their bowels, frequently of their own accord mentioned both the headache and the constipation. Rarely there was an early diarrhea. The older patients frequently suffered almost unbearable pain in the frontal region, "unlike anything previously experienced." Often the degree of prostration was so extreme that it was difficult to obtain a history. The extremely toxic or the bulbar types were lethargic and stuporous, sometimes irrational, and usually irritable and hyperesthetic when aroused. A few were unduly alert. Cerebral manifestations such as diplopia, blurring vision, dysarthria, vertigo, and incoordination of muscles were common. These early cerebral symptoms frequently presaged a high involvement of the nervous system while those with lower extremity pains

*From the Communicable Disease Service, Unit No. 1, Los Angeles General Hospital.
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frequently terminated with lower cord lesions. An extreme sensitiveness to slight stimuli was very noticeable. Deep muscle pains, especially in adults, was often unduly acute. Sometimes these pains made their appearance long after the fever and other systemic symptoms had subsided (posterior poliomyelitis). Urinary retention was occasionally present early but rarely persisted as the pathology of poliomyelitis does not involve the sympathetic system. Many cases started very insidiously with mild symptoms and a few showed focal symptoms almost as soon as any illness was recognized.

Examination was often entirely negative at this stage, although stiffness of the neck with acute pain along the spine when anteflexed was a common early finding. Whether this be due to stretching of the spinal muscles or to tension on the dura of the cord, it is a suggestive sign of early central nervous system invasion and frequently precedes the positive spinal fluid findings. Hyperactivity of both deep and superficial skeletal reflexes was an early neurologic finding, although the latter were frequently absent when the patient was first seen. Occasionally nystagmus or other evidences of cranial nerve irritation were noted in the systemic stage.

The average case in this series first presented itself at the hospital on the fourth day of illness and at that time every case showed some degree of fever. The maximum was 105° F. with an average of 102. Frontal headache was complained of in 70 per cent of cases although many small children and unintelligible foreigners gave no subjective history. Neck rigidity was present in 95 per cent of cases although pain in the neck and lumbar region was mentioned by only 32 per cent of patients. Pain on anteflexion of the spine however, was an almost constant finding, and was almost invariably accompanied by Brudzinski's sign. On admission, 18.3 per cent presented localized pain in one or more extremities. The severity of the headache probably distracted attention in many cases from the milder pains elsewhere. Gastrointestinal symptoms were as follows: constipation 92 per cent, diarrhea 2 per cent, nausea 60 per cent, vomiting 40 per cent, pain in abdomen 3 per cent, difficulty in swallowing 0.86 per cent. Urinary findings included incontinence in 8 cases and retention in only one. Only three cases of the 350 showed convulsions or gave history of same. Fifty and two tenths per cent showed some localized paresis and 11.7 per cent showed definite paralysis on admission.

Following the invasion stage the usual sequence of symptoms followed. The actual number of the abortive type was difficult to estimate because of the uncertainty of the diagnosis in cases not developing central nervous system symptoms. Sixty per cent made complete recoveries before leaving the hospital, and this obviously included some of the 50 per cent who showed localized pareses on admission. A rather unusual observation among the dromedary types was the occasional occurrence of pains and paresis as long as three weeks after the initial preparalytic onset and after an interval of two weeks of freedom from all symptoms. Some of these patients had been sent home with diagnosis other than poliomyelitis. In those cases which made a gradual transition from the systemic stage to that of the central nervous system invasion, the first symptoms were usually pain and weakness of the affected part. Asymmetry of the reflexes was the first objective evidence, and frequently the only deciding factor on which to base a

diagnosis. Peculiarly, however, the abdominal, spinal, or gluteal muscles frequently showed the earliest reflex changes but rarely any residual paralyses. As is usual in poliomyelitis, none of our patients showed residual bladder or bowel paralyses.

Of the various laboratory procedures only the spinal fluid findings were of diagnostic significance. The usual changes were: a slight increase in pressure of from 2 to 10 mm. of mercury; slight increase in globulin; increased cell count (mostly lymphocytes); and a characteristic colloidal-benzoin precipitation test. Occasionally the spinal fluid was entirely unchanged, especially in the mild infections, and a diagnosis then had to be made on clinical findings alone. Also, in the early systemic or preparalytic phase of the disease the spinal fluid findings were frequently negative and misleading, and we learned in questionable cases to defer puncture (but not treatment) until some clinical evidence of neurologic involvement, principally changing or asymmetrical reflexes or painful anteflexion of the spine, was present. Quite early in the epidemic a few cases diagnosed "upper respiratory infection," etc., on the strength of negative spinal fluid findings later developed muscular paresis. Thereafter these doubtful cases were given intravenous or intramuscular serum and the diagnosis later confirmed by subsequent puncture or positive clinical findings. This procedure raised the question whether or not convalescent serum, if given to normal individuals, might not itself be responsible for spinal fluid changes, but a few patients thus treated who made uneventful recoveries (probably not poliomyelitis), were later tapped and their spinal fluids found to be entirely negative, thus nullifying this contention. In our series 70 per cent of spinal fluids were under increased pressure, and cell counts ranged from 0 to 1083 per c.mm., with an average of 87 cells per patient. Differential counts showed lymphocytes predominant in 95 per cent of cases, polymorphonuclears in 3 per cent and an equal distribution of both types in the remaining 2 per cent. In 79 per cent of cases, the colloidal-benzoin test was typical, i.e., negative precipitation in the first five tubes, maximum precipitation in the second five, and decreased or absence in the last five. It was totally negative in 8.6 per cent, showed the tuberculous meningitis type of curve in 8.6 per cent of cases, was "atypical" in 0.8 per cent and questionable or not reported in 3 per cent. *In* 12.86 *per cent of cases the spinal fluids were negative cytologically but the patients showed characteristic neurologic findings,* while 22 per cent of cases were negative neurologically (abortive type) but showed conclusively positive spinal fluids. Blood counts in our series ranged from 6,000 to 18,000 with an average of 13,000 and 79 per cent polynuclears, obviously not alone characteristic of poliomyelitis. In only a few cases where diagnoses were doubtful were blood cultures or blood chemistry determinations done, and these were all negative or of no positive diagnostic value in favor of poliomyelitis.

The diagnosis of the disease in its early stages is difficult and often impossible. An obvious toxemia, particularly if there is undue prostration, and the cardinal symptoms of frontal headache, fever, stiffness, and pains in the back and in the neck aggravated by anteflexion, and some degree of gastrointestinal upset accompanied by constipation, is almost diagnostic of the preparalytic phase, particularly in the presence of an epidemic. If to the above is added evi-

dence of central nervous system involvement, especially asymmetrical or changing reflexes, unilateral weakness, pains, or paresthesiae, the suspicion is strengthened. If the spinal fluid then shows an increased pressure, globulin, or increased cell count with a preponderance of lymphocytes, the diagnosis is almost certain. Poliomyelitis, if seen in the preparalytic stage, must be differentiated from all acute infectious diseases, particularly acute upper respiratory or gastrointestinal toxemias. Of the former, the epidemic (streptococcus) sore throats were the most common in our series, although several cases of ordinary coryza and sinusitis, and a few cases of influenza (questionably abortive poliomyelitis), otitis media, bronchitis, and pneumonia were seen. Two cases of pyelitis, in which the local symptoms were overshadowed by the systemic, were admitted to the hospital as suspects; also several cases of hysteria. Encephalitis, although rare, was seen in a few instances at this time, although here again the question of etiology (bulbar involvement) was present. Meningitis, particularly the tuberculous type, was frequently seen and, rarely, confused for a short time with the bulbar type of the disease. A purely clinical differentiation between tuberculous meningitis, encephalitis and bulbar poliomyelitis is frequently most difficult if not impossible, and in these diseases the colloidal-benzoin test has been of great help. Osteomyelitis was seen for differentiation in several instances, and also an occasional case of multiple neuritis (especially alcoholic), acute rheumatic fever, central nervous system syphilis, brain abscess, and cerebral accident. One case each of meningococcus septicemia, acute endocarditis with embolic phenomena and acute reticuloendotheliosis were seen during this epidemic. In infants, birth injuries, scurvy, rickets, and congenital muscular weakness were all forwarded to the service for differentiation from poliomyelitis. One case of poliomyelitis was complicated by the presence of scurvy. The diagnosis in some few cases was, and always will remain a mystery, but in a majority of instances the ultimate findings were conclusive. In time of epidemic, a communicable disease hospital always receives a large number of cases for differentiation from those of the prevailing illness.

There were many toxemias. Some of these patients recovered spontaneously and others later showed localizing symptoms. Of the spontaneous recoveries only those showing cytologic changes in the spinal fluid were considered as abortive poliomyelitis. Those with paresis were obviously all poliomyelitis, but, as above stated, *and contrary to current medical opinion,* 12.86 *per cent showed no cytologic changes in the spinal fluid.* Of those patients with obvious sources of their toxemia (particularly the septic sore throats), and who developed no neurologic findings, only those were considered as positive whose spinal fluids showed cellular changes. All of these fluids also showed typical colloidal-benzoin curves. This test we believe to be of greatest relative value. Toxemias are common in many diseases and the "meningismus" often seen is as much a part of the systemic manifestations of the disease as the headaches or the skeletal pains. That changes should occur in the spinal fluid in such cases is not inconceivable, and in a few instances we did spinal punctures on patients with various other acute infections for the purpose of evaluating the colloidal-benzoin test. All showed some degree of precipitation but no cellular changes, and these were classified as extreme toxemias involving the central nervous system and not as abortive polio-

myelitis. Irrespective of etiology, poliomyelitis is an anatomic and pathologic entity, and localizing symptoms, formerly discussed, when clearcut, justify us in so diagnosing the case.

The hospital treatment of poliomyelitis during this epidemic was largely with pooled convalescent human immune serum, plus the usual rest, immobilization of affected extremities, dietetic and eliminative measures. Occasionally methenamine was rather empirically used because of its supposed diffusion into the spinal canal, and calcium for its muscle tonic effect was likewise given with about the same degree of expectancy. Hypertonic saline intravenously was used in a few cases when serum was not available. Previous experience with Rosenau's serum has caused us to abandon its use, and the one patient in this series who received it before admission to the hospital, developed the all too common allergic reaction, a terrific urticaria. The amount of convalescent serum given averaged 15 c.c. per patient when given intrathecally, and 30 c.c. when given intramuscularly or intravenously. The choice of method was determined by the stage of the disease, the severity of onset, the certainty of diagnosis, and evidence of central nervous system involvement. It has been definitely established that dyes given intramuscularly and intravenously do not appear in the spinal fluid for at least twenty-four hours, and we feel very strongly that if the toxin has reached the central nervous system as evidenced by neurologic and spinal fluid findings, that the antidote should be given intrathecally as well as intravenously, in order to neutralize the toxin in its most dangerous site without unnecessary delay. Similarly, we feel that when the disease is still a toxemia without central nervous system phenomena, that the intravenous or intramuscular routes may be the most logical. Having had experience with intracisternal therapy in this hospital, where over 1,000 punctures have been done in the past year for the treatment of various conditions, we have generally preferred this approach when giving serum intrathecally. The criticism of intrathecal medication because of irritant preservatives in the serum seems illogical in view of the splendid results obtained with antimeningococcic, antitetanic, and other sera given this way, all of which contain preservatives and which also have the added disadvantage of being horse sera to which a patient may show an allergy. Reactions to medication were shown in only five of our cases and these were very mild and transient.

While it is difficult to estimate the value of any therapeutic measure in this disease, which is too serious to permit of untreated controls, and which normally includes such a high percentage of spontaneous recoveries, nevertheless our results seem to warrant our method of treatment. The intracisternal injection of serum seemed to be the turning point in the febrile stage of the disease in 82 per cent of the cases, and the subjective symptoms likewise seemed to rapidly abate following its administration. We were able in one instance, to definitely arrest a case of the ascending or Landry's type with intracisternal medication, a precedent in this hospital. The number of complete recoveries (60.9 per cent); the 25.66 per cent of cases with very mild residual paresis, all of whom will probably recover fully; the 10.28 per cent of residual paralyses most of whom are improving; and the low death rate of 3.16-per cent, point to a mild type of the disease, early recognition, or efficient treatment. The death rate for Los Angeles

County during the same epidemic, as given by the Health Department, was over 7 per cent. In as much as this figure is for the same community and the same epidemic but includes patients treated outside the hospital, we feel that our treatment must have been a factor in the low death rate within the institution. Judging from data on previous epidemics in this community, there is no reason to believe that the disease was milder in 1930 or in this locality than elsewhere. Our results, as contrasted with methods outlined in California and Western Medicine for October, 1930, have been accomplished with very much smaller doses of serum; not that we may not have desired in many instances to have given more, but because the inadequate supply required conservation; and from the results, the dosages used, seemed ample. We feel that the relatively low morbidity rate here was largely due to the cooperative efforts of the city and county health authorities and the private physicians resulting in early recognition of cases and their rational treatment.

REFERENCE

1. Aycock, W. L., and Kramer, S. D.: Immunity to Poliomyelitis in Normal Individuals in Urban and Rural Communities as Indicated by Neutralization Test, J. Prev. Med. 4: 189-200, 1930.

CARBON MONOXIDE ACUTE AND CHRONIC POISONING AND EXPERIMENTAL STUDIES*

By Thomas L. Ramsey, M.D., and H. J. Eilmann, M.A., Ph.D., Toledo, Ohio

Carbon Monoxide Affinity for Hemoglobin; Acute and Chronic Poisoning; Experimental Studies of Effect of Automobile Exhaust Gas on Guinea Pigs

HEMOGLOBIN is very widely distributed throughout the entire animal kingdom, being found in the blood corpuscles of mammalia, birds, reptiles, amphibia and fishes; it is also found in the blood corpuscles of many of the invertebrates. The composition of its molecule varies somewhat in the different animals, so that, strictly speaking, there are probably a number of different kinds of hemoglobin, all, however, closely related in chemical and physiologic properties.

Hemoglobin has the property of uniting with oxygen in certain definite proportions, forming a true chemical compound, known as oxyhemoglobin. Each molecule of hemoglobin unites with a molecule of oxygen. Oxyhemoglobin is not a very firm compound. If placed in an atmosphere containing no oxygen it is dissociated, giving off free oxygen and leaving behind hemoglobin or so-called reduced hemoglobin. This power of combining with oxygen to form a loose chemical compound, which in turn can be dissociated easily when the oxygen pressure is lowered, makes possible the function of hemoglobin in the blood as the carrier of oxygen from the lungs to the tissues.

There are a number of pigmentary bodies which are formed directly from hemoglobin by decomposition or chemical reactions of various kinds. The best known and most important are: methemoglobin, nitric oxide hemoglobin and Carbon Monoxide hemoglobin.

METHEMOGLOBIN

This pigment is closely related to oxyhemoglobin, since it contains the same amount of oxygen and is isomeric with it. The oxygen is, however, not in loose combination and cannot therefore be utilized by the system.

NITRIC OXIDE HEMOGLOBIN

Nitric oxide forms a firm compound with hemoglobin rapidly oxidizing it to methemoglobin.

CARBON MONOXIDE HEMOGLOBIN

This pigment is a molecular combination of one molecule of carbon monoxide with one molecule of hemoglobin. It forms a stronger combination than oxygen and hemoglobin, carbon monoxide having an affinity for hemoglobin approximately three hundred times that of oxygen. Since carbon monoxide readily

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combines with both oxyhemoglobin as well as with reduced hemoglobin, the tissues suffer for want of oxygen. Carbon monoxide hemoglobin imparts to the blood a bright, cherry red color both in the venous and arterial circulations and can be readily recognized by a number of standard tests.

CARBON MONOXIDE

Carbon monoxide is a colorless, tasteless, inodorous gas. Its most common sources are coal stoves, grates, salamanders, domestic and industrial furnaces, gas engines, coal, natural, and artificial gases. It is formed whenever incomplete combustion of carbon occurs or, as a matter of fact, anywhere where combustion of the carbon takes place slowly with an insufficient quantity of oxygen. The first stage of combustion produces the poisonous gas CO. A second stage then produces comparatively harmless gas, CO_2. In the first stage each carbon atom unites with one atom of oxygen, in the second stage with two atoms of oxygen.

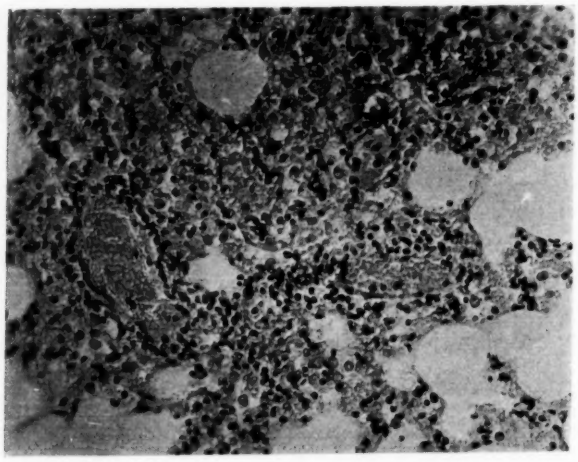

Fig. 1.—Guinea pig lung (×175). Shows the hyperemia and the localized areas of consolidation. These changes are not unlike those found in any section of guinea pig lung and are not specific for carbon monoxide poisoning.

In a coal fire the process may be different, the combustion taking place in the lower layers of red hot coals may be complete but the CO_2 passing upward through the less heated layers may give up some of its oxygen or take up more carbon, forming carbon monoxide.

In burning buildings carbon monoxide may be produced in large amounts and is the chief constituent of the smoke which overcomes the firemen. In fires and explosions in mines it is the carbon monoxide that often causes more deaths than the explosion or fire itself.

Tobacco smoke from cigarettes contains considerable carbon monoxide: McNally[1] found that the carbon monoxide from inhaled smoke was from 0.014 to 0.26 per cent of the tobacco and paper consumed; from cigars it was from 0.027 to 0.15 per cent and from pipe tobacco 0.027 per cent. This averages about 80 c.c. of carbon monoxide to each gram of tobacco burned.

The proportion of carbon monoxide differs greatly in domestic and industrial gases. The commercial gas served to the consumer, which is a mixture of gases

from wood, coal and naphtha, contains about 11.25 per cent of carbon monoxide. An atmosphere containing 0.2 per cent is capable of destroying life. Haldane.[2]

AUTOMOBILE EXHAUST GAS

The internal combustion engine where a mixture of air and gasoline vapor is caused to burn produces carbon monoxide in the exhaust varying anywhere from 3 to 10 per cent. The richer the mixture, the more of the CO gas produced. As a rough figure it has been estimated that one cubic foot of CO gas is produced per minute for each 20 horsepower of the motor. From this one can estimate how quickly a small garage can be polluted to a concentration sufficient to overcome a person in the enclosure and to produce death in a short time.

Analysis of street air has shown carbon monoxide in varying amounts but never in sufficient amount to be distinctly poisonous. The gas escaping from the

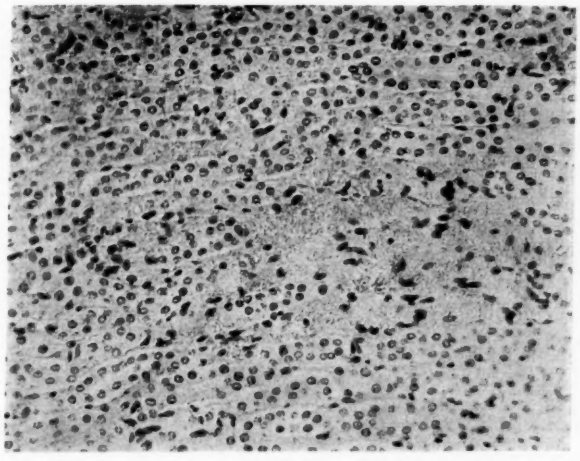

Fig. 2.—Guinea pig kidney (×100). Acute CO death; shows active hyperemia and a small hemorrhagic area.

automobile exhaust is rapidly diluted by the surrounding air so that one standing behind a car not in motion but with the engine running, would be in an atmosphere of about 4 parts to 10,000. Carbon monoxide concentration in the air must exceed 15 parts per 10,000 to produce possible serious results.

The atmospheric concentration times the time of exposure determines the amount of absorption. Police officers on traffic duty in the large cities have shown as high a concentration as 30 per cent saturation of the hemoglobin with carbon monoxide. The New York Division of Industrial Hygiene in 1923 in a survey of 157 garages, repair shops and service stations found carbon monoxide positive in 69.5 per cent of the workers and carbon monoxide was present in the air in appreciable amounts in 77.5 per cent of the places examined.

Henderson[3] in a study of the exhaust system for the New York, New Jersey vehicular tunnel made the following observations:

1. When the time of exposure in hours times the concentration of the CO in parts per 10,000 equals 3, there is no appreciable effect.
2. When the result is 6 there is just a perceptible effect.

3. When the result is 9 there will be headache and nausea.
4. When the result is 15 or more the conditions are dangerous to life.

ACTION

Carbon monoxide may be freely respired, causing no irritation of the air passages, but the moment it comes in contact with the blood by diffusion, it unites with the hemoglobin forming carbon monoxide hemoglobin.

Hill and Barcroft[4] have determined that CO combines more readily with unsaturated oxyhemoglobin than with hemoglobin, in other words, hemoglobin will take up more CO at a given tension if a little oxygen is present than if oxygen is completely absent.

Nicloux[5] has shown that the red blood cells even when saturated with carbon monoxide are not devitalized but are ready to resume functioning when supplied

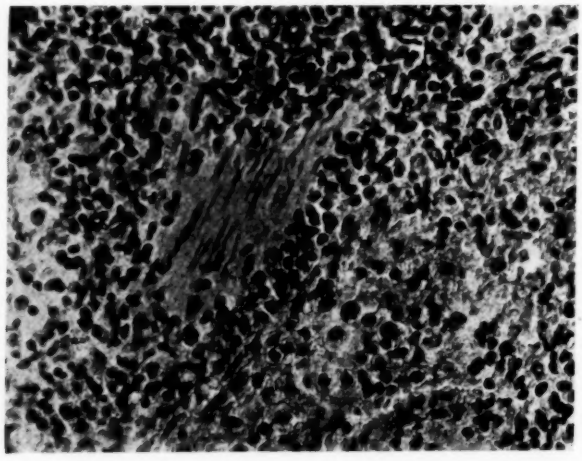

Fig. 3.—Guinea pig spleen (×175). Acute CO death; pulp space lower right-hand corner filled with blood, active hyperemia.

with oxygen. Blood containing CO hemoglobin may be deprived of the gas by submitting it to diminished pressure or by passing air or oxygen through it for a considerable length of time.

The development of poisoning from carbon monoxide depends entirely upon the concentration of the CO in the air and the time of exposure. The higher the concentration of CO in the inspired air, the greater the saturation of the blood with corresponding decrease in the time of exposure. For instance: 0.02 to 0.03 per cent CO in the inspired air will only cause a 23 to 30 per cent saturation of the blood in five to six hours whereas 0.5 to 1.0 per cent CO will cause a 73 to 76 per cent saturation of the blood in two to five minutes.

SYMPTOMS AND EFFECT UPON THE BODY

The symptoms depend upon whether the poisoning is acute or chronic. In the acute cases the symptoms depend upon the concentration of the CO in the inspired air and progress according to the degree of concentration present.

As anoxemia progresses the blood pressure is at first increased as a result of reflex stimulation of the vasomotor center, later the pressure is decreased due

to a benumbing of this center and a dilatation of the blood vessels. Apoplexy may occur in the first stage. The pulse becomes slower as the blood pressure rises; the heart beats violently; subsequently the pulse becomes frequent but small. The breathing is deep and difficult as a result of deficient oxygenation and the diminished production of CO_2. Headache, throbbing of the temples, tinnitus, faintness, dizziness, vertigo and even vomiting may occur. The face becomes flushed, more or less extensive patches of bright red color make their appearance on the surface of the body. The muscular system is quite early affected, special muscle groups may become paralyzed, there may be tonic or clonic spasms. Involuntary urination and defecation may occur. The body temperature is lowered, unconsciousness may occur early which is especially true when ethane gas is present with the CO. Death occurs when the respiratory center is paralyzed. This usually occurs when the blood becomes saturated to about 70 to 80 per cent.

Fig. 4.—Guinea pig liver (×175). Photograph taken using blue filter to bring out portal capillaries; shows active hyperemia around central vein.

If removed in time recovery sometimes takes place rapidly, but more usually symptoms persist for some time. Muscle paralysis and even degeneration may continue. Sensations to pain may remain absent for a long time.

Various opinions may be found in the literature as to the action of CO upon the nervous system and other body tissues. The inhalation of oxygen with 20 per cent CO has been reported to have caused cramps and total paralysis within the first minute of inhalation which could certainly not have occurred because of the anoxemia itself. Kobert,[6] Geppert,[7] and others strongly incline to the belief in its action upon the nervous system, both on the peripheral nerves and on the ganglion cells of the brain, and they extend the poisonous action of the gas to the production of a degeneration of the muscles and glands.

Haggard[8] has demonstrated that CO has no direct toxic action upon the nervous system. Haldane[2] and his coworkers have generally concluded that CO unites only with hemoglobin and that it owes its toxicity solely to the fact that it thus interferes with the oxygen carrying power of this compound. It is possible, however, that it unites with other oxygen receptors as well as those of the

hemoglobin and it may thus act directly on cells. It is found to be somewhat more toxic, especially toward the basal ganglia of the brain, than equivalent asphyxia for mammals, which would seem to bear out this view.

Other sequelae that have been mentioned are primary gangrene, blisters, decubitis, persistent distention of the capillaries, pneumonia, and deep seated disturbances of degeneration of all the organs, especially of the vascular walls and ganglion cells.

CHRONIC CARBON MONOXIDE POISONING

Chronic carbon monoxide poisoning occurs as a result of breathing small quantities of carbon monoxide over long periods of time. Digestive disturbances, diminished vigor, coated tongue, loss of memory, more or less muscular weakness are all reported as occurring. Some workers have reported anemia, simulating

Fig. 5.—Guinea pig brain cortex (×175). Shows considerable edema, pyramidal zone normal.

the pernicious type, others including Davis (quoted by Forbes),[9] Haines[10] have reported cases of polycythemia, sometimes the count running as high as six to nine million with a hemoglobin above normal. It is claimed that the increase in the red cells is a protective effort on the part of the system.

Gruber[11] has shown that CO is not a cumulative poison, when inhaled in very small amounts it rapidly disappears from the blood. Therefore the blood examination may not be an aid to diagnosis in chronic cases.

TREATMENT

Much could be said in regard to the prevention of CO poisoning. The incidence of this form of poisoning far exceeds that of all other forms of poisoning combined and yet it is surprising how little action has been taken to prevent its occurrence. By this I mean, legislative action.

In some states regulations have been passed and rigid inspections of the sources of origin of the gas are carried out. Education of the public in this matter seems to be considerably neglected, and it is only occasionally that it is

called to one's attention by reading of certain deaths having occurred from this source.

During a three-year period, 1918, 1919 and 1920, there occurred in New York City and Chicago alone 2916 deaths from poisoning, such as wood alcohol, bichloride of mercury, carbolic acid, potassium cyanide, strychnine, narcotics, carbon monoxide, etc. Out of these 2916 deaths 2298 were caused by carbon monoxide. In these same cities there were 3167 deaths from poisoning during 1928 and 1929, and 2628 were due to carbon monoxide poisoning.

Carbon monoxide asphyxia, if untreated, may continue in force for a long time even after the patient is removed from the poisonous atmosphere. Early inhalation treatment to assist in the elimination of this gas from the blood is essential. Oxygen 93 per cent with carbon dioxide 7 per cent, seems to be the inhalation of choice. The carbon dioxide acts as a stimulant to the respiratory

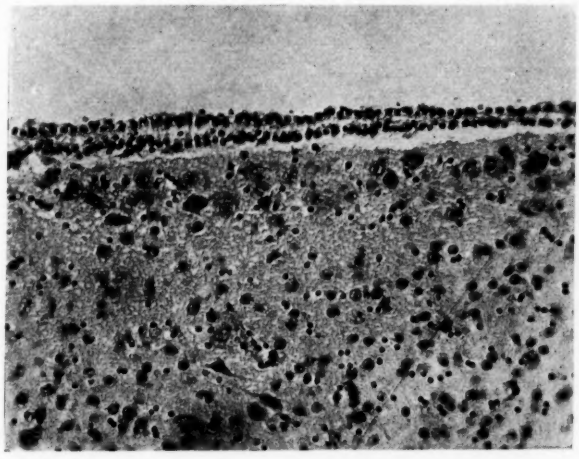

Fig. 6.—Guinea pig brain, floor of ventricle basal ganglion (×175). Some edema, ganglion cells normal, no hemorrhage.

centers. If respiration has stopped, artificial respiration should be given by the prone pressure method and continued until it is seen to be useless or success is attained. If the carbon dioxide is not available, oxygen alone or even fresh air is of value. The victim should not be moved in severe cases except to remove him to a fresh air atmosphere. Warmth should be applied and it is important that the victim be placed upon warm rugs or garments and not on the cold floor or ground.

Hypodermic medication is useless. When the patient can be moved he should be taken to his home or to a hospital for observation and care. Blood transfusion is also valueless, probably due to the fact that CO combined with tissue receptors becomes released and enters the infused corpuscles converting the oxyhemoglobin to carbon monoxide hemoglobin. Until the carbon monoxide is largely eliminated from the body cells, this process would occur.

Until the patient is fully restored to normal he should be kept absolutely quiet and not even allowed to sit up. The length of time necessary for elimination of the gas varies, of course, with the amount absorbed and the treatment received.

There seems to be quite a variance of opinion as to the rapidity of its elimination, some claim that this change may take place in a couple of hours, others state in from four to six hours and others that the gas is eliminated quite slowly. It is unquestionably eliminated more rapidly if oxygen or oxygen and carbon dioxide are administered, but it is very questionable whether this gas is eliminated so rapidly by medium of the air alone. Our experiments have convinced us thoroughly that this is the case.

Out of 43 consecutive cases received at the Cook County Hospital at Chicago,[12] 34 per cent were examined within one half hour after the patient was removed from the source of exposure; CO was found in all. In 30 per cent the exact time was not known but was greater than one half hour, 27 per cent being positive and 3 per cent were negative. In 14 per cent of the cases twenty to forty minutes had elapsed and CO was present in 12 per cent and negative in 2

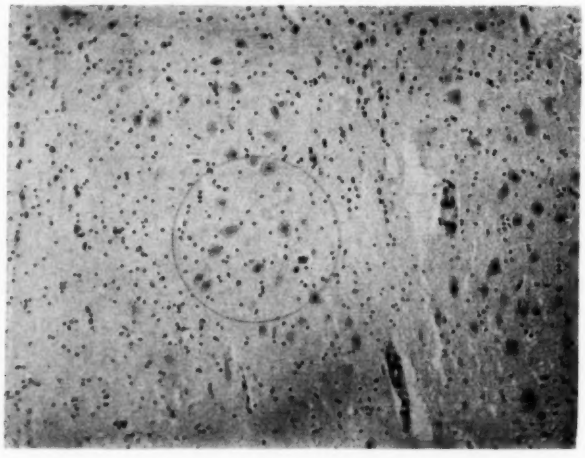

Fig. 7.—Guinea pig brain, medulla (×100). Showing ganglion cell areas, normal.

per cent. Of the 10 per cent examined after three hours all were positive; in another 10 per cent five hours had elapsed, all were positive, and in 2 per cent twelve hours had passed and all gave positive tests. The negative cases found in this series may not have been exposed to quantities of the gas and may have been unconscious from other substances such as ethane which occurs in illuminating gas.

It has been claimed that CO may be in part changed by oxidation into CO_2 within the body. Gruber[11] and others claim that this oxidation does not take place but that the CO is voided, quantitatively, unchanged. Whatever process of elimination occurs, it is certain that it is not rapid. The fact that this gas has an affinity for hemoglobin approximately 200 to 300 times that of oxygen would support this claim. On account of the variance of opinions a series of experiments were performed to make a study of this question and to observe the pathologic changes that occurred from inhalation up to varying percentages of blood saturation. A series of guinea pigs were used and a special box, air-tight with full glass top and measuring 4 by 3 by 2 feet, with an inlet for the gas and

a corresponding outlet for the air, and with a door for handling the animals, was designed and used, obtaining the CO from the exhaust of an automobile by a connection of rubber tubing. The CO was found to be 8 per cent with the motor running easily. This remained quite constant.

TABLE I

EXPERIMENTS*

(Guinea Pigs)

NO.	CO CONCENTRATION	FIRST SYMPTOM	COLLAPSE AFTER	REMOVED AFTER	DEATH AFTER	CO% IN BLOOD	NO. OF DAYS CO FOUND IN BLOOD
1	gas started	1 min.	3 min.		10 min.	70% plus	
2	full sat.	at once	40 sec.		5½ min.	70% plus	
3	full sat.	50 sec.	2 min.		5¾ min.	70% plus	
4	full sat.	30 sec.		2 min.	3½ min. later	70% plus	
5	full sat.	50 sec.	1½ min.	1½ min.	Recovered	60%	31 days
6	full sat.	30 sec.	1½ min.	1½ min.	Recovered	55%	25 days
7	full sat.	30 sec.		1 min.	Recovered	40%	10 days
8	½ sat.	1 min., 40 sec.	5 min.		14 min.	70% plus	
9	½ sat.	1½ min.	7 min.		24 min.	70% plus	
10	½ sat.	1½ min.		4 min.	Recovered	60%	28 days
11	½ sat.	1 min., 40 sec.		3 min.	Recovered	55%	20 days
12	½ sat.	1 min., 40 sec.		2 min.	Recovered	40%	15 days
13	¼ sat.	2 min., 30 sec.		4 min.	Recovered	50%	8 days
14	¼ sat.	2 min.		3 min.	Recovered	30%	5 days
15	¼ sat.	no symptom		2 min.	Recovered	25%	3 days

*The animals were placed in a special gas chamber connected by tube to the exhaust of an automobile and were observed through a glass top. Special sliding doors were arranged so that they could be removed at any time without disturbing the CO concentration.

The pigs were exposed to atmosphere fully saturated, 50 per cent and 25 per cent saturated. Table I is a summary of this series of experiments.

EXPERIMENTAL OBSERVATIONS

From these experiments it was seen that the pigs that died all showed at least 70 per cent saturation of CO in the blood. In atmospheres not fully saturated where the animal is allowed to breathe the CO somewhat longer, the blood concentration is high when the animal is removed and lives. It is also evident that CO does not rapidly disappear from the blood but is slowly eliminated.

Another series of experiments were performed upon dead animals to determine the ability of the hemoglobin to absorb CO after death.

In 4 guinea pigs killed by etherization, laked blood saturated with CO from illuminating gas, was injected into the muscles, also beneath the skin and into the peritoneal and thoracic cavities. Twenty-four hours later the animals were autopsied and all showed similar appearances to those that had died from inhalation of this gas. This same experiment was performed on two pigs killed by etherization and then injected with embalming fluid. Twenty-four hours later laked blood with CO was injected and the results were practically the same.

Two guinea pigs killed by etherization and embalmed for twenty-four hours were placed in an atmosphere of illuminating gas. These pigs had been previously autopsied and the thoracic and abdominal cavities were open. After

eight hours the tissues and all the blood showed marked evidence of saturation with CO.

Two other pigs were killed by etherization and embalmed for twenty-four hours and not autopsied but, with the skin intact, were placed in an atmosphere of illuminating gas, contents of the jar being changed by allowing the gas to enter once an hour for eight hours. The pigs were then kept in the sealed jar overnight. Upon examination of these animals they presented the typical appearances of all the other animals that had died by inhalation of the gas.

These points are of considerable importance from a medicolegal standpoint and were suggested by the experiments of Strassmann and Schultz[13] who demonstrated that CO may penetrate by diffusion all parts of a cadaver with sufficiently long exposure to air containing this gas. It also brings out the fact that if a body has been buried in a region where gases containing carbon monoxide

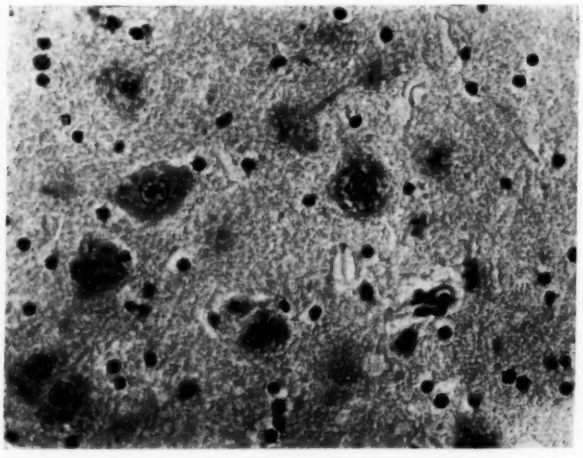

Fig. 8.—Guinea pig brain, medulla ganglion cell area (×375). Same as encircled area in Fig. 9. Normal ganglion cells.

are present in the earth, this gas may penetrate the body and be present in the blood in sufficient quantities to lead to an erroneous conclusion that death had been caused by carbon monoxide poisoning. It is also evident that a body may be so tampered with before or after autopsy that an erroneous conclusion may be formed and death attributed to carbon monoxide poisoning.

POSTMORTEM APPEARANCES

The body may present little evidence as to the cause of death, but generally well marked cherry red blotches will be seen on the dependent parts of the trunk, neck and thighs. The eyes are usually closed, the countenance usually composed.

Upon opening the body one is immediately struck by the unusual bright cherry red color of the muscles and of the venous as well as the arterial blood. The blood is usually fluid and is present in the arteries, veins and all of the tissues. The brightness of the blood may be masked by carbon dioxide when it has also been present in the inspired air. When the action of the gas has not been so concentrated and the exposure longer, small hemorrhages may be present in some

of the tissues together with pulmonary edema and a bright red froth in the air passages. The gastric and intestinal mucosa may also show small punctate hemorrhages. The kidneys and liver show few if any changes. Glycosuria has been present in about 20 per cent of the cases that have been examined.

When life has been prolonged, the skin may show blebs, herpes and even gangrene. Some of the muscles may show degeneration. If the patient has lived

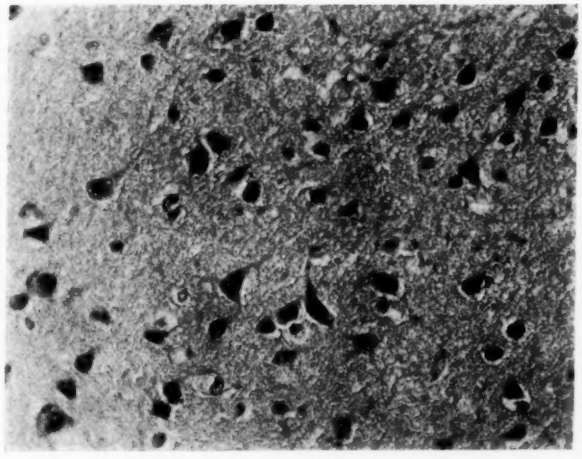

Fig. 9.

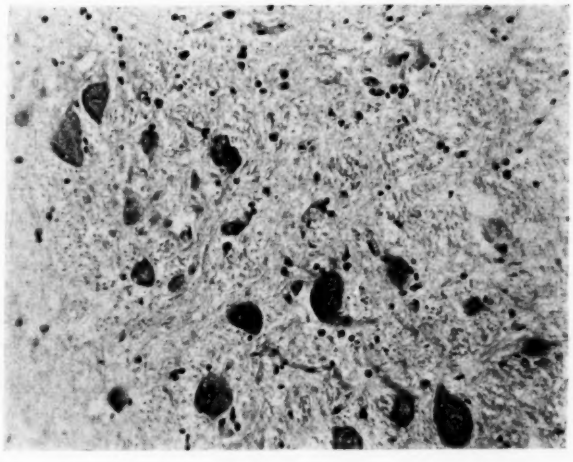

Fig. 10.

Figs. 9 and 10.—Areas showing ganglion cells from human brain of acute CO death, normal.

for a number of hours only a careful examination of the blood may reveal the presence of the gas. It must also be remembered that the blood is bright red in color when death is due to cyanide poisoning, but then the color is not so permanent and changes to blue upon exposure to the air. Carbon monoxide can be readily recognized by a number of standard tests, principally the sodium hydroxide, the potassium ferrocyanide, the tannin tests, and spectroscopic ex-

amination. Another point of interest from a medicolegal as well as diagnostic standpoint is the fact that formaldehyde and embalming fluid do not interfere with the determination of CO in the blood; Tollens[14] even recommends adding some formaldehyde to the blood solutions when making spectroscopic examinations.

POSTMORTEM PATHOLOGY. MICROSCOPIC

In order to determine the effect of carbon monoxide on the body tissues, sections were made from all the pigs that died during the experiment and from several that lived for varying lengths of time following exposure. The parts sectioned included cerebral cortex, basal ganglia, medulla, lungs, heart, liver, spleen, kidneys, suprarenal glands and voluntary muscle.

The tissues from the animals that died during the experiment or immediately afterwards presented the following findings:

Cerebral cortex: in the majority the findings indicated edema, some congestion of the pial vessels and occasionally minute hemorrhages.

Basal ganglia: moderate edema, no apparent congestion of vessels, no hemorrhages were present.

Medulla: no variation from the normal with the possible exception of some edema. No hemorrhages and no degenerated ganglion cells were seen.

Lungs: edema, congestion of capillary vessels, areas of minute hemorrhages, with some filling of peribronchial air cells and a moderate amount of exudate especially in the smaller bronchial tubes.

Heart muscle: showed no variation from the normal.

Liver, spleen, kidneys and suprarenal glands: all of these tissues appeared normal with the exception of occasional minute hemorrhages and congestion of capillary vessels. The most marked changes were in the kidneys where minute hemorrhages were more frequent in the cortical zone.

Voluntary muscle: none of the sections showed any evidence of hemorrhage or muscle cell degeneration, all sections appeared normal.

The tissues from the animals that lived after varying lengths of exposure showed no variation from the normal with the exception of the lungs. Evidence of emphysema, peribronchial consolidation and bronchial exudation were present in all. The brain tissues and ganglion cells, heart muscle and voluntary muscle showed no evidence of degeneration. All the organs sectioned showed no changes other than those usually found in pigs sectioned after death by etherization.

These microscopic findings hardly substantiate the claims presented in other reports.

SUMMARY

We have presented a study of carbon monoxide from a standpoint of the method of its production, its affinity for hemoglobin, its action upon being respired, the percentages of blood saturation in varying lengths of time according to the concentration in the respired air, the symptoms and effect upon the body, possible methods of its elimination from the body, the production of chronic carbon monoxide poisoning, methods of treatment, the postmortem macroscopic appearances of the body following carbon monoxide deaths, some important

medicolegal facts, a series of experiments on guinea pigs to ascertain its persistence in the body following exposure, and the histopathology of various tissues in animals dying, directly during exposure, and those killed and autopsied at varying periods later.

CONCLUSIONS

We admit that this is but a preliminary step in any study of this interesting subject but it does seem from our findings that CO is not rapidly eliminated from the body unless other measures than simple respiration of air are used.

It appears that in acute cases death is caused entirely by anoxemia and respiratory failure.

The body after death is capable, even after embalming, of absorbing sufficient carbon monoxide, when concentrated and with sufficient time of exposure, to produce all of the macroscopic appearances and positive chemical findings of death due to this gas.

It is also possible to produce similar findings by injecting the body with either laked or whole blood, saturated with CO. These possibilities are of grave medicolegal importance.

REFERENCES

1. McNally, Wm. D.: quoted in "Legal Medicine & Toxicology," Peterson, Haines, Webster **2**: 297, 1923.
2. Haldane, John: Carbon Monoxide, J. Physiol. **18**: 430-462, 1895.
3. Henderson, Yandell: The Physiological Principles Governing Ventilation When the Air Is Contaminated With Carbon Monoxide, J. Indust. & Eng. Chem. **14**: 229-236, 1922.
4. Hill, Archibald V., and Barcroft, Joseph: The Combinations of Hemoglobin with Oxygen and With Carbon Monoxide, Biochem. J. **7**: 470-491, 1913.
5. Nicloux, Maurice: Intoxication aigue oxycarbonique survie traitement par l'oxygene pur et dosage de l'oxyde de carbon dans le sang pendant la periode de retour, Presse méd. **29**: 701-703, 1921.
6. Kobert, E. R.: Lehrbuch **2**: 871, 1906.
7. Geppert, A. J.: Quoted in Kobert's Lehrbuch **2**: 871, 1906.
8. Haggard, H. W.: Studies in Carbon Monoxide Asphyxia. The Growth of Neuroblast in the Presence of Carbon Monoxide; a Demonstration That This Gas Has No Direct Toxic Action Upon Nervous Tissues, Am. J. Physiol. **60**: 244-249, 1922.
9. Carbon Monoxide, Legal Medicine & Toxicology, Peterson, Haines, Webster **2**: 309, 1923. 1923.
10. Haines, Walter S.: Carbon Monoxide, Legal Medicine & Toxicology, Peterson, Haines, Webster, **2**: 309, 1923.
11. Gruber, Max: Ueber den Nachweis und die Giftigkeit des Kohlenoxyds und sein Vorkommen in Wohnraeumen, Arch. f. Hyg. **1**: 145, 1883.
12. Cook County Hospital Reference, quoted in Legal Medicine & Toxicology, Peterson, Haines, Webster **2**: 302, 1923.
13. Strassmann, Fr., and Schultz, A.: Untersuchungen zur Kohlenoxydvergiftung, Berl. klin. Wchnschr. **43**: 1233-1237, 1904.
14. Tollens, B.: Ueber Blut-Spectralreaction bei Gegenwart von Formaldeheyd, Ber. d. deutsch. chem. Gesellsch. **34**: 1426-1427, 1901.

ENDOGENOUS URIC ACID AND HEMATOPOIESIS*

III. Uric Acid Outputs and Reticulocyte Counts as Affected by Glycine, Caffeine, Urea, Bilirubin, Atophan, and Xylose

By Joseph Krafka, Jr., Augusta, Ga.

MARKED increases in uric acid outputs have been demonstrated by the author (Krafka, 1929, 1930), following regeneration of the red blood cells after severe hemorrhage and after hemolysis. The reticulocytes, or young red cells, present in the peripheral circulation have been numerically correlated with the uric acid outputs. These results support the hypothesis that relates endogenous uric acid to the nucleoprotein derived from the destruction of the extruded nuclei of the normoblasts at the maturation of the erythrocytes.

In a search for the factor responsible for the maintenance of the balance between red cell production and red cell destruction, a check has been made on the various substances known to increase uric acid elimination, such as glycine, caffeine, atophan, and several simple diuretics, together with bilirubin and xylose. The last two are considered because of their relation to red cell destruction in the one instance and nuclear destruction in the other.

EXPERIMENTAL

The majority of the tests were carried out on a pure-bred Dalmatian coach dog. The complete data include daily observations on erythrocyte counts, reticulocyte counts and uric acid outputs for 239 consecutive days. The more significant results were checked on a series of rabbits and one test was carried out on a human being. All drugs were administered by mouth except in cases indicated.

The methods of testing are essentially those used by other investigators, that is, by establishing a base level as a control before administration of the drug. The data are presented in table form.

Glycine.—Since Lewis, Dunn, and Doisy (1918), Christman and Mosier (1929), have tested the action of glycine on uric acid outputs, two tests were carried out on our dog. Three grams of glycine (Eastman Kodak Co.) were fed February 17. The uric acid output for the subsequent twenty-four hours was 727 mg. as compared to 324 mg., average for the four previous days. A second dose of 6 grams of glycine on February 18, however, was followed by a uric acid output of only 400 mg. The rise was not consistent for the second dose. The results are best explained as due to the increased elimination since the first dose brought out a total urine volume of 640 c.c., while the second produced only 425 c.c. A similar relationship may be seen in the two investigations cited above, particularly in the three experiments of Christman and Mosier.

*From the University of Georgia, Medical School, Department of Anatomy.
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In our test the reticulocytes rose from 32,500 per c.mm. to 52,110 on the subsequent day and dropped again to 6,020, 11,640, 22,520. There was no proportional rise on the third day as would have been expected had a real marrow stimulation taken place. On our hypothesis, our data favors the view that glycine increases uric acid outputs by elimination only. The rise and fall in reticulocyte count is explained on the basis of flushing the current crop prematurely, as will be discussed more at length under the tests with caffeine.

The data are given in Table I.

Urea.—Under the hypothesis of a specific dynamic action of amino acids, Lewis, Dunn and Doisy have tested urea. The dosage which they used had only a very slight diuretic effect and showed no increase in uric acid output. Our initial dose of 20 grams was also evidently subminimal, since no diuresis was obtained and no increase in uric acid was noted over the prevalent level. However, the second dose of 75 grams brought out 1060 mg. on a volume of 1350 c.c. at a

TABLE I

EFFECT OF INGESTION OF GLYCINE UPON URIC ACID OUTPUTS AND RETICULOCYTE COUNTS OF THE DALMATIAN COACH DOG

	RETICULOCYTES PER C.MM. OF BLOOD	DAILY URINE VOLUME C.C.	DAILY URIC ACID OUTPUT IN MG.	FED
Feb. 14	35,700	200	320	
15	39,760	310	352	
16	27,150	450	328	
17	27,400	275	312	3 grams glycine
18	52,110	640	727	6 grams glycine
19	6,020	400	400	
20	11,640	280	320	
21	22,520	510	500	
22	10,400	270	432	

TABLE II

EFFECT OF INGESTION OF UREA UPON URIC ACID OUTPUTS AND RETICULOCYTE COUNTS OF THE DALMATIAN COACH DOG

	RETICULOCYTES PER C.MM. OF BLOOD	DAILY URINE VOLUME IN C.C.	DAILY URIC ACID OUTPUTS IN MG.	FED
Dec. 31	33,920	810	405	
Jan. 1	17,040	450	300	
2	42,280	380	432	75 grams urea
3	41,020	1315	1060	
4	34,710	350	400	
5	51,030	360	320	
6	34,980	350	400	

time when the base level was 377 mg. There was no subsequent reticulocytosis, and hence increased elimination of uric acid is again indicated (Table II).

Soda Bicarbonate.—A dose of 20 gm. of sodium bicarbonate also produced a marked diuresis and with it flushed out a large quantity of uric acid. A volume of 2380 c.c. carried with it 1260 mg. uric acid. This occurred on a low blood count of 3,710,000 on an actively regenerating marrow, and hence the influence on reticulocyte count cannot be determined directly.

Ammonium Chloride.—A series of consecutive doses of ammonium chloride showed somewhat similar results. Six grams given May 27 produced 830 c.c. of

urine and 744 mg. of uric acid; 16 gm. the following day produced 2075 c.c. of urine and 1208 mg. of uric acid; 8 gm. on the next day produced 650 c.c. of urine and 750 mg. of uric acid. This was in a period of active regeneration with a blood level of 3,520,000 on May 26 to 4,860,000 on May 30. During this period no marked rises in reticulocytes occurred which might be attributed to the action of the salt, the level being maintained at 14,000 to 41,000.

One dose of lithium citrate and one dose of uvi ursi were also given but without any diuretic effect.

Caffeine.—Eight doses of caffeine were administered under a series of conditions. The first dose of 450 mg. produced a urine volume of 550 c.c. and 550 mg. of uric acid. The second dose was evidently subminimal, since the volume dropped to 315 c.c. and 168 mg. of uric acid. The third dose, given three days later in three portions of 300, 300 and 450 mg., resulted in a urine volume of 475 c.c. and 672 mg. of uric acid. The fourth dose was 600 mg., given on a blood level of 4,000,000 and just after a spontaneous uric acid peak of 675 mg., due to

TABLE III

EFFECT OF INGESTION OF CAFFEINE ON URIC ACID OUTPUTS BY THE DALMATIAN COACH DOG

	FED	DAILY URINE VOLUME IN C.C.	DAILY URIC ACID OUTPUT IN MG.	R.B.C.
Jan. 9	450 mg. caffeine	460	368	
10	300 mg. caffeine	550	550	5,820,000
11		315	168	
12		470	248	
13	1050 mg. caffeine	325	288	
14		475	632	
23		675	675	
24	600 mg. caffeine	500	400	4,020,000
25		300	344	
29		370	424	
30	1200 mg. caffeine	600	650	3,940,000
31		1470	1170	
Feb. 1		440	584	
26		400	456	
27	1200 mg. caffeine	560	448	6,180,000
28		1750	439	
Mar. 7		600	480	
8	1200 mg. caffeine	570	648	5,870,000
9		1670	835	
10		290	376	
23*		875	776	
24	1200 mg. caffeine	915	808	5,030,000
25		1460	1064	

*Period of placental feeding, hence the high uric acid base level.

active erythrocyte regeneration. This may explain the drop to 344 mg. of uric acid with a volume of 300 c.c. after the caffeine. The next four doses are of 1200 mg. caffeine each, and marked diuresis was obtained in each case. The fifth dose, on a low blood count of 4,000,000 and an active marrow, brought out 1170 mg. of uric acid in a urine volume of 1470 c.c. The sixth dose, given on a blood count of 6,000,000 and after three high daily outputs due to the ingestion of nucleic acid, produced a marked diuresis of 1750 c.c. but with an extremely low

uric acid content of 439 mg. The seventh dose, on a blood level of 5,870,000 and a low reticulocyte count, resulted in a urine volume of 1670 c.c. and 835 mg. of uric acid. The eighth dose was followed by 1460 c.c. of urine and 1064 mg. of uric acid, but the last value is of no significance since it occurred in a period of active regeneration, low blood count, and placental feeding.

It is of interest to note that in the experiments reported by Myers and Wardell (1928) initial doses of caffeine always acted as diuretics and the largest outputs of uric acid came down with the largest volumes. They seem to favor the hypothesis that the caffeine is converted into uric acid, principally, because of the fact that while theobromine is a good diuretic, it failed to increase the uric acid outputs in their four tests. An examination of their data shows that the dosage used failed to act as a diuretic. Their tests with euphylin show definitely that fluid intake and output were increased and that the uric acid increased proportionately. Our data seem to indicate that caffeine increases uric acid outputs by diuresis since the amounts are correlated with the condition of the marrow at the time.

The only outstanding fact against this explanation is the observation of Clark and Lorimer (1926) that the blood uric acid rises simultaneously with increased outputs under caffeine feeding.

The reticulocyte behavior under the conditions of caffeine administration throws further light on this explanation, and at the same time presents some evidence as to the mechanics of erythrocyte delivery.

Reticulocytosis Induced by Caffeine.—In six of the eight tests of caffeine, a marked reticulocytosis was produced. The increases, however, occurred on the day subsequent to the administration of the drug, and in this way differed from the spontaneous rises observed after hemolysis and hemorrhage, which followed at intervals of from three to four days after uric acid peaks, as reported in an earlier paper (Krafka, 1930).

The first dose of 450 mg. caffeine, brought out 64,020 reticulocytes at a time when the prevailing level was 38,010. With a higher prevailing level, caffeine flushes out proportionately more reticulocytes. Thus the fifth dose of 1200 mg. brought out 304,850 reticulocytes, when the count on the previous day was 149,720. The values for the other doses are given in Table IV. Proportional increases of 1.7 to 3.5 times were observed. The second and fourth doses present exceptions.

TABLE IV

EFFECT OF INGESTION OF CAFFEINE UPON THE RETICULOCYTE COUNTS IN THE DALMATIAN COACH DOG

	CAFFEINE ORAL DOSE IN MG.	RETICULOCYTE COUNTS: PREVAILING	AFTER CAFFEINE	SUBSEQUENT	COEFFICIENT OF INCREASE
Jan. 9	450	38,010	64,020	39,500	1.7
10	300	64,020	39,500	33,480	
13	1050	28,900	75,140	34,500	2.5
24	600	100,500	104,000	98,560	
30	1200	149,720	304,850	55,480	2.0
Feb. 27	1200	31,400	77,550	12,240	2.5
Mar. 8	1200	17,610	63,000	5,400	3.5
24	1200	155,930	287,190	78,820	1.8

It will also be noted from Table IV, that a plethora followed the increase. This is particularly evident in the massive 1200 mg. doses. The fifth dose showed a drop to 56,480 from a preexperimental level of 149,720.

The data may be interpreted as due to the flushing action of the caffeine by increasing the vascular pressure. The entire reticulocyte crop is released into the general circulation instead of being retained for the maturing period of three to four days. A subsequent plethora follows.

These observations are of considerable interest in connection with the mechanics of erythrocyte delivery. Drinker, Drinker and Lund (1922) have shown that it is impossible to wash out nucleated red cells by increased mechanical pressure. In our eighth test with caffeine a check was made on this point, and it was found that there was no increase in the relative number of circulating normoblasts, although the reticulocyte count rose from 155,930 to 287,190. There is thus a marked observed difference in the mechanics of the delivery of these two types of cells.

This point is of such interest that the test was repeated on a series of ten rabbits. The data are given in Table V.

It is apparent from Table V that caffeine increases the reticulocyte counts, particularly after the regenerative process has begun after hemolysis. This point is well brought out in the test of June 11, 1931. Counts were made in the morning, caffeine administered at noon, and recounts were made in the afternoon, with increases consistent throughout.

The increases are not marked or regular on an inactive marrow, a fact also observed in the Dalmatian hound.

Atophan (Phenylcinchonic Acid).—Since this drug has been used rather extensively in gout, its relation to uric acid outputs has been widely studied. Our two tests failed to show anything of significance. An initial dose of 250 mg. given at a time when the erythrocyte count was 6,100,000, reticulocytes at 0.3 per cent and uric acid at 560 mg., resulted in a subsequent R.B.C. of 5,880,000, reticulocytes 0.2 per cent and uric acid 664 mg. A second dose of 750 mg. failed to change the reticulocyte count or materially influence the uric acid output; the values being 584 mg. before administration and 500 mg. after.

Bilirubin.—From time to time, the theory has been developed that the by-products of hemoglobin destruction may act in the capacity of erythropoietic agents, capable of maintaining the nice balance between destruction and production of erythrocytes. Verzar and Zih (1929) claim to have obtained marked increases in the red cell count by the oral administration of small doses of bilirubin and biliverdin. This observation was of such interest in an alternate hypothesis developed by the author, that a test of bilirubin was made, checking not only the red cell count but also the reticulocyte count and the uric acid values.

The initial dose of 3.5 mg. of bilirubin was given when the erythrocyte count was 5,210,000; reticulocytes 0.4 per cent; uric 310 mg. The subsequent day, the red cell count was 4,870,000; reticulocytes 0.5 per cent and the uric acid output 288 mg. A second dose of 3.7 mg. of bilirubin was given with the R.B.C. at 4,700,000; reticulocytes at 0.1 per cent and uric acid at 424 mg. The result was

TABLE V

EFFECT OF CAFFEINE ON THE RETICULOCYTE COUNT IN RABBITS

RABBIT	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	NO. 9	NO. 10
5/21	5,150,000 1.4	4,710,000 4.1	5,450,000 1.0	5,470,000 1.2	5,130,000 2.7	5,310,000 2.7	4,280,000 1.7	5,690,000 0.8	5,380,000 2.6	5,150,000 1.3
5/22	4,950,000 0.9	5,110,000 2.9	5,500,000 1.0	5,530,000 2.5	5,360,000 1.9	5,070,000 2.2	4,920,000 0.8	5,490,000 0.2	4,780,000 2.4	4,980,000 2.1
5/23	5,130,000 1.9	4,980,000 4.3	5,180,000 1.7	5,200,000 0.7	5,200,000 2.2	5,100,000 2.2	4,120,000 2.2	5,100,000 0.8	5,270,000 4.3	5,470,000 2.1
5/26	9:50-11:15 A.M. 50 mg. caffeine citrate in water intravenous. Counts 11:20-4:12 P.M.									
	5,290,000 1.2	5,360,000 2.7	5,250,000 1.9	5,040,000 1.9	5,040,000 2.1	4,920,000 3.1	4,690,000 2.3	4,360,000 1.7	5,080,000 2.2	5,190,000 1.2
5/28	9:00-10:00 A.M. 100 mg. caffeine citrate by mouth. Counts 2:35-4:14 P.M.									
	5,380,000 1.7	5,190,000 2.9	5,340,000 2.3	5,310,000 2.0	5,360,000 2.0	4,690,000 4.7	5,220,000 2.0	5,400,000 3.4	4,750,000 4.5	5,440,000 2.2
6/3	9:00-10:00 A.M. 100 mg. caffeine (plain) by mouth. Counts 3:00-4:40 P.M.									
	5,650,000 2.4	5,380,000 4.9	5,560,000 3.1	5,710,000 3.5	5,710,000 3.7	5,080,000 4.9	5,710,000 3.4	5,950,000 4.8	4,970,000 4.5	5,290,000 2.5
6/5	(9:30-10:00 A.M. 50 mg. phenylhydrazine HCl by mouth.)									
6/6	4,780,000 1.7	4,660,000 2.8	4,560,000 2.4	4,360,000 2.6	4,600,000 1.2	4,660,000 2.7	4,450,000 2.9			
6/9	10:00 A.M. 50 mg. caffeine orally. (Counts at 2:30-4:00 P.M.)									
	3,450,000 9.0	3,700,000 8.6	3,830,000 6.6	3,000,000 17.3	3,380,000 9.7	3,870,000 7.8	3,200,000 8.2			
6/10	11:00 A.M.									
	3,160,000 1.6	3,900,000 2.4	4,240,000 2.7	3,390,000 9.3	3,350,000 5.9	4,360,000 3.4	3,010,000 7.0			
6/11	10:00 A.M.									
	3,780,000 4.8	3,660,000 5.6	4,510,000 4.8	3,540,000 10.3	3,440,000 4.2	4,020,000 5.7	3,340,000 6.7			
	(50 mg. caffeine orally at 12:00 N. Counts 2:00-3:00 P.M.)									
	10.9	8.8	10.0	22.2	15.8	7.7	12.5			

insignificant since the erythrocytes were 5,100,000; reticulocytes 0.7 per cent and the uric acid level 320 mg. A third dose of 3.6 mg. gave similar results.

Verzar and Zih do not report reticulocyte counts in their work and until marked increases in this group of cells are demonstrated, bilirubin cannot be considered an active hematopoietic agent.

Xylose.—As a correlary to the hematogenous theory of the origin of endogenous uric acid, a series of tests were carried out on the by-products of nuclear destruction in an attempt to find a factor which might control the production of erythrocytes by its varying concentrations. Uric acid was the first substance tried. A massive dose of 1500 mg. was given a patient with a severe secondary anemia without materially improving the red cell count or the reticulocyte count.

Larsell, Jones and Phillips (1927) have reported hematopoietic effects from the intravenous injection of nucleic acid and nuclear extractives in both rabbits and human beings. Dakin, Howe and West (1931) have identified the active substance in liver extract as a hydroxyproline. It seemed possible then, that somewhere in the splitting up of the nucleoprotein of the extruded normoblast nucleus, an active agent might be found. Of the three principal derivatives of nucleic acid, phosphoric acid seemed the least promising, since its concentration in the blood may be varied by so many independent processes. The pentoses, purines and pyramidines remained, and two of these have been tested. On the basis of the report by Dakin, Howe and West that the precipitate containing both hypoxanthine and pentose from liver extract were inactive in the treatment of pernicious anemia, and that the active substance is free from pyramidines, our own limited tests seem to indicate that nuclear derivatives are not concerned directly with the erythropoietic mechanism.

A test of pentose was carried out on the Dalmatian hound, by first depressing the erythrocyte count by hemolysis with phenylhydrazine and then feeding 5 gm. doses of xylose, observing the regenerative rate as shown by the erythrocyte and the reticulocyte counts. This was not in excess of regenerative rates spontaneously observed for the same animal in earlier tests. The data are given in Table VI.

The xylose was from cottonseed hulls purified by three recrystallizations by Dr. C. H. Maryott.

It is of interest to note that Madders and McCance (1930) report that the ingestion of 5 gm. doses of xylose or arabinose have no effect on the uric acid outputs in man.

Adenosine Compounds.—Drury and Gyorgyi (1931) have extracted a substance from freshly minced bullock's heart by 5 per cent trichloracetic acid which has a marked action on the heart of the dog when injected into the circulation. They have identified this substance as adenosine. Using the same method of extraction, we have checked the effect on reticulocyte counts in two rabbits, but without showing any activity on the marrow. We further checked the activity of the extract on a cat under urethane anesthesia and obtained a sharp fall in blood pressure upon the injection of 1 c.c. The right femoral artery was ligated before the injection; the marrow of the right and left femora was fixed in formalin and sections prepared. No observable differences in the marrow of the two legs were found.

These observations are in keeping with those of the authors cited above that subcutaneous doses of 50 mg. daily had no effect on the erythrocyte counts in guinea pigs.

TABLE VI

EFFECT OF INGESTION OF XYLOSE UPON THE RETICULOCYTE COUNTS IN THE DALMATIAN COACH DOG

	ERYTHROCYTE COUNT PER C.MM. BLOOD	PER CENT RETICULOCYTES	FED
May 12	4,710,000	0.1	
13	4,670,000	0.1	
14	5,120,000	0.2	
15	5,260,000	1.3	50 mg. phenylhydrazine HCl
16	4,560,000	0.5	
18	3,970,000	1.5	
19	3,900,000	1.7	5 gm. xylose
20	3,910,000	1.0	5 gm. xylose
21	3,900,000	1.9	5 gm. xylose
22	4,150,000	2.1	10 gm. xylose
23	4,420,000	2.2	
25	4,500,000	0.5	
26	5,030,000	3.2	

SUMMARY

1. Glycine, urea, sodium bicarbonate, and ammonium chloride increase the daily outputs of uric acid primarily by their diuretic action.

2. Caffeine markedly increases the output of uric acid apparently through its diuretic action, although the amounts vary with the regenerative states of the marrow.

3. Caffeine produces a marked reticulocytosis the degree of which is dependent upon the marrow activity. It differs in time from the spontaneous reticulocytosis in that it follows the immediate administration of the drug.

4. Caffeine does not increase the number of circulating normoblasts while the reticulocytosis is produced; hence a marked difference exists in the mechanics of delivery of these two types of cells.

5. Minute doses of bilirubin failed to act as hematopoietic agents as checked by reticulocyte counts.

6. Xylose failed as a hematopoietic agent as checked by reticulocyte counts.

7. Adenosine also failed as a hematopoietic agent as checked by reticulocyte counts and by the histologic picture of the marrow.

8. The data are considered in their relation to the theory that relates endogenous uric acid to the extruded nuclei of the normoblasts.

REFERENCES

Christman, A. A., and Mosier, E. C.: Purine Metabolism: II. The Effect of Ingestion of Glycine on the Excretion of Endogenous Uric Acid, J. Biol. Chem. **83**: 11, 1929.

Clark, G. W., and Lorimer, A. A.: The Effects of Caffeine and Theobromine Upon the Formation and Excretion of Uric Acid, Am. J. Physiol. **77**: 491, 1926.

Dakin, H. D., Howe, M., and West, R.: A Precipitant for Material in Liver Active in Pernicious Anemia, Proc. Soc. Exper. Biol. & Med. **28**: 512, 1931.

Drinker, C. K., Drinker, K. R., and Lund, C. C.: The Circulation in Mammalian Bone-Marrow, Am. J. Physiol. **62**: 1, 1922.

Drury, A. N., and Gyorgyi, A. Z.: The Physiological Activity of Adenine Compounds With Special Reference to Their Action Upon the Mammalian Heart, J. Physiol. **68**: 213, 1929.

Krafka, Jos., Jr.: Endogenous Uric Acid and Hematopoiesis, J. Biol. Chem. **83**: 409, 1929.

Endogenous Uric Acid and Hematopoiesis. II. Uric Acid, Reticulocytes, and Erythrocytes After Hemolysis by Phenylhydrazine Hydrochloride, J. Biol. Chem. **86**: 223, 1930.

Larsell, O., Jones, N. W., and Phillips, B.: The Hematopoietic Effect of Intravenously Injected Nucleic Acids, J. A. M. A. **89**: 682, 1927.

Lewis, H. B., Dunn, M. S., and Doisy, E. A.: Studies in Uric Acid Metabolism. II. Protein and Amino Acids as Factors in the Stimulation of Endogenous Uric Acid Metabolism, J. Biol. Chem. **36**: 9, 1918.

Madders, K., and McCance, R. A.: Effect of Pentose Ingestion on Uric Acid Excretion, Biochem. J. **23**: 1175, 1930.

Myers, V., and Wardell, E. S.: Influence of Ingestion of Methyl Xanthines on the Excretion of Uric Acid, J. Biol. Chem. **77**: 697, 1928.

Verzar, F., and Zih, A.: Die Hämopoëtische Wirkung von Bilirubin und anderen Hämoglobin Derivaten, Biochem. Ztschr. **205**: 388, 1929.

GINGIVITIS: CHEMOTHERAPEUSIS AS AN AID IN THE DIAGNOSIS AND TREATMENT*

Preliminary Report

By Herbert Marshall Cobe, Ph.D., Philadelphia, Pa.

A FRESHLY isolated bacteria will, when subjected to the action of various antiseptics, either be killed completely (bacteriolysis), be retarded (bacteriostasis), or grow as on ordinary nutrient agar, or other media. The antiseptic coefficient of standard drugs has no relation to the different organisms, as different strains of the same species react to the same agent in a markedly different manner.

Based on the fact that members of the same group of bacteria and even different strains of the same organism have a diversity of reactions to a standard chemical, it is seen that they exhibit degrees of resistance which are independent of the type of drug used, but more dependent on the phase of their development. They do not actually build up a so-called "immunity" to the drug but they manifest a varying degree of penetrability to the reagents and develop a tolerance to it. This degree of tolerance is definitely associated with morphologic and attenuation changes. Consequently, in freshly isolated, rapidly developing and dividing strains this tolerance is less than in those strains which have been or are exhibiting advanced phases of microbic dissociation. Just as bacteria develop a resistant or "R" form to the continual lysis of the bacteriophage, so does a strain of bacteria develop a similar type of resistance to the continued use of an antiseptic.

It is this fact which explains a condition very frequently found in the treatment of gingivitis. A definite point or plateau is reached in treatment and from which no amount of medication with previously successful antiseptics has any effect. The lesion settles into one of chronicity with possibly periodic exacerbations of an acute nature. Or, as in the case of Vincent's infection, there is frequent recurrence.

Chemotherapeusis is in reality, the adaption of suitable chemicals or drugs to the treatment for elimination of diseases and disease condition. These drugs and antiseptics are the most standard preparations of various drug houses. They are sold and standardized in relation to their (1) antiseptic powers, (2) penetrative ability, (3) safety and convenience in usage.

The preparations most frequently in use in oral hygiene and for the treatment of gingival infections are divided into three main groups: (a) mercurials, (b) salts of other metals, and (c) dyes. With these, a chemotherapeutic standard is used of two strong antiseptics whose coefficient of antisepsis has been accepted; one is phenol itself (in a final dilution of 1-1,000), the other is tricresol (final dilution of 1-2,000). These groups of germicides give us a range of the common

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antiseptics covering most of the solutions used in ordinary dental or medical practice for the treatment of gingivitis.

The technic followed is the same one used and described by Keilty,[1] the only variation is in the concentration of the work done on the streptococci isolated from gingival cultures by anaerobic as well as aerobic methods. Keilty's results have been so uniformly good that any variation would only slow up the treatment. The entire work depends on a cooperation between the laboratory and the dentist to give a maximum of efficient diagnosis and treatment.

Technic.—

A. Two direct smears are made for staining:

1. For the usual stain by Gram's method,
2. For differential stain if wanted.

B. A suspension of the material, fresh, in salt solution for a dark-field examination or with simple oil immersion lens, to note motility, character of the spirilla, protozoa, type and number of organisms.

C. A suspension in Schaudinn's fluid for ameba present.

D. A blood agar plate is inoculated for media control and initial culture from which subcultures are made for further bacterial identification.

E. Tubes of the special antiseptic agar are inoculated by stroke and stab to determine the degree of growth allowed by the different antiseptics.

The antiseptic tubes are all made up as follows: Nutrient agar is melted and flasked in 50 c.c. lots, to this is added 2 per cent blood collected aseptically and 0.5 c.c. of a 1-100 dilution of the antiseptic to be tested. This gives in the final dilution an antiseptic agar of 1-10,000, in all flasks. This is then tubed in small tubes and used directly after slanting. The standards (phenol and tricresol) are made up in corresponding proportions to give the final dilutions as desired.

The following preparations are the ones made up as being the ones most commonly used by contributing dentists. We have discarded some and are adding others. For example, antiseptics which persist in showing growth in a dilution of the antiseptic of 1-10,000, and in dilutions of greater concentrations, which generally show growth are not suitable clinically where the dilution in the mouth is greater in a few moments than that which we are using in each test tube. Among those which gave consistently poor results are: S.T.37, and mercurochrome. These have been discarded from the set up and we are confining the test to eleven antiseptics. They are:

Mercurials	Metaphen (Abbott Laboratories) Merthiolate (Eli Lilly) Mercurochrome (discarded)
Salts of metals Oxidizing agents	Copper sulphate Chromic acid Argyrol Iodine, acetone, glycerine (Prinz Formula)
Analine dyes	Acriviolet Gentian violet
Standards	Tricresol Phenol

TABLE I
22 STRAINS OF ORGANISMS INHIBITED BY ANTISEPTICS

ORGANISMS		MERCURIALS		DYES		ARGYROL
		METAPHEN	MERTHIOLATE	GENTIAN VIOLET	ACRIVIOLET	
Gram-negative rods	B. coli communis	G	NG	G	NG	G
	B. coli communior	G	NG	G	G	G
	B. lactis aërogenes	G	NG	G	G	G
	M. catarrhalis, 3 strains	3 NG	2 NG	G	1 NG	G
Gram-negative cocci	B. mucosus capsulatus	NG	NG	G	G	G
	S. salivarius (2 strains)	2 NG	2 G	2 G	2 NG	G
	S. pyogenes	2 NG	3 G	3 G	3 NG	1 G
	Strept. subacidis	G	NG	G	G	G
Streptococci	S. Hemolyticus I	G	G	G	NG	G
	S. fecalis	G	G	G	NG	G
	S. anginosus	G	G	G	NG	NG
	S. mitior	G	G	G	NG	NG
	S. equinus	G	G	G	G	G
	S. aureus hemolyticus (3 strains)	G	3 NG	1 NG	2 NG	G
Other gram-positive cocci	M. tetragenus	G	G	G	NG	G
	Pneumococci	G	NG	G	NG	G
	Diptheroids	G	NG	G	NG	G

There is a definite relationship between the degree of infection and the type of organism present. In the most severe infections, there is a predominance of streptococci, and these respond less to treatment than do the other types of infection. Gram-negative organisms are less difficult to clear up than the gram-positive ones. The opinion has been prevalent that the gram-positive organisms respond better to drugs which have as a basis one of the coal tar products; i.e., the dyes, and that the gram-negative organisms respond best to mercurials. This is not wholly in accord with our findings. The streptococci respond equally well to the mercurials as to the dyes, but there is a slightly greater affinity for the gram-negative rods and the mercurials. A mixed flora, however, might react differently to two of the individual strains and require still a third antiseptic to act on the combination of the strains.

Table I shows the results of the isolation of single strains of bacteria by culture. When isolated pure, they were then put through all of the chemotherapeutic media. The streptococci are classified according to Holman,[2] based on their fermentation reactions. All the organisms were isolated from gingival sulci except the strains of the colon group which were obtained from cases of mucous

colitis. The antiseptics which gave retarded growth are not included in the table, growth however feeble was recorded as being equal to no inhibition.

In inoculating slants, two main methods were tried in order to get a method for comparative results. The first was to inoculate a tube of Rosenow's deep brain broth from a pus pocket. After twenty-four hours' incubation a loopful of the broth culture was transferred to the antiseptic tubes and incubated overnight. This gave varying results but there was doubt as to all of the organisms present in the pus pocket surviving, as it is well known that there is inhibition of one type of organism by another. Therefore slants were inoculated directly from the gingival sulcus or pocket. In badly infected cases this generally gave the same amount of material transferred to each tube; generally a loopful. Following is the result of fifty cases so inoculated:

TABLE II
FIFTY CASES OF GINGIVITIS, CHEMOTHERAPEUTIC RESULTS

ANTISEPTIC	RETARDED	PER CENT	NO GROWTH	PER CENT
Chromic acid	4	8	0	0
Copper sulphate	2	4	0	0
Merthiolate	6	12	17	34
Mercurochrome	0	0	0	0
Metaphen	12	24	10	20
Argyrol	0	0	2	4
Gentian violet	4	8	0	0
Acriviolet	4	8	19	38
Phenol	14	28	10	20
Tricresol	14	28	6	12
I. A. G.	3	6	4	8

When growth occurred the antiseptic was considered as being a failure, even though the growth was markedly restricted. In Table II a report is made of this retardation but is merely placed there as a matter of record for comparative purposes.

The percentage of nongrowth produced by the different cultures of the gingiva should determine the percentage of its efficiency. Table III shows the percentage of each of the antiseptics used in relation to their bacteriolytic efficiency.

TABLE III
PERCENTAGE CHART OF ANTISEPTICS USED

SOLUTION	PER CENT OF EFFICIENCY
Acriviolet	38
Merthiolate	34
Metaphen	20
Phenol	20
Tricresol	12
Iodine, Acetone, Glycerine	8
Argyrol	4
Chromic acid	0
Copper sulphate	0
Gentian violet	0
Mercurochrome	0

Gingival cultures show a diversified bacterial flora. In Table IV there is a high percentage of streptococci and M. catarrhalis. The unexpectedly large percentage of moulds found is noteworthy:

TABLE IV

BACTERIAL INCIDENCE IN 100 CASES OF GINGIVITIS

ORGANISMS	PER CENT OF CASES	ORGANISMS	PER CENT OF CASES
M. tetragenus	24	S. aureus	44
B. mucosus capsulatus	19	S. albus	6
Diphtheroids	29	S. citreus	1
Pneumococci	76	S. hemolyticus	17
B. influenzae	9	S. nonhemolyticus	15
M. bucallis	2	S. infrequens	12
Streptothrix	48	S. salivarius	33
Leptothrix	81	S. pyogenes	12
M. catarrhalis	73	Protozoa*	75

*Trichomonas buccalis 36 per cent.
Endameba buccalis 39 per cent.

The application of the germicide is an important factor. It is immaterial how it is applied if one is certain that the bacteria and the germicide are in contact. For this purpose a flexible rubber vacuum cup has been recommended. This holds a sufficient quantity of solution to be time-saving in application. There is no doubt also, that there are benefits derived from the vigorous massage given by the use of this cup. Pus pockets of depth are reached by the use of a bent, blunt platinum needle with a syringe. The method of application is secondary if the fact be borne in mind that any germicide must be in contact with the organisms to be effective.

In treatment the alternate use of the suggested drug has been combined with arsphenamine in some form or other. This is used to clear up the spirochetes which we have found to be present in 86 per cent of all normal mouths, and in 98 per cent of all those infected with one or more of the streptococcus group. It is doubtful whether the type of arsenical can be used indiscriminately, but the results with neoarsphenamine and sulpharsphenamine in glycerine, applied topically, have been remarkably good when used with the various antiseptics. These arsenic preparations are definite spirocheticides and clear up the spirilla present.

SUMMARY

Chemotherapy is definitely indicated in gingival infections. The cooperation of the laboratory and dentist is necessary in suggesting the proper antiseptic to use in treatment of gingival infections such as, streptococcal gingivitis, ulceromembranous gingivitis, pyorrhea, etc.

Gingival infections due to gram-negative organisms respond to treatment better and more rapidly than do those due to gram-positive organisms. There seems to be no basis for the idea prevalent in medical minds that the gram-positive organisms respond more successfully to the dyes than to the mercurials.

Gingival cultures show a diversified bacterial flora with a large percentage of moulds and 89 per cent of streptococci present in lesions. For chemotherapeutic diagnosis inoculation by stroke and stab is necessary and direct inoculation gives the most accurate results. Merthiolate, metaphen, acriviolet, and phenol were found to be the four antiseptics of highest bacteriolysis of eleven used in a series of fifty cases.

In treatment the use of two or more of the bacteriolytic antiseptics used alternately and in combination with arsphenamine has been found to give the most satisfactory results and with fewer recurrences.

REFERENCES

1. Keilty, R. A.: Specificity of Bacteria to Bacteriolytic Action of Chemicals With Note on This Application to Chemotherapy, J. Lab. & Clin. Med. **14**: 539, 1929.
2. Holman's Classification of Streptococci, J. Med. Research **29**: 377, 1916.

MERTHIOLATE AS A SKIN DISINFECTING AGENT*

By H. H. Marks, M.D., H. M. Powell, Sc.D., and W. A. Jamieson, A.B.,
Indianapolis, Ind.

INTRODUCTION

MANY studies have been published upon the use of various germicides as human skin sterilizing agents.[1, 2, 3, 4, 5, 6, 7, 8] In some instances in the literature on this subject, artificial conditions have been introduced, such as animal skin disinfection tests, the use of laboratory cultures as well as certain contaminated materials artificially applied to the skin, and experiments dealing only with the very superficial bacteria associated with the skin. Such tests do not necessarily indicate the true effectiveness of a germicidal agent against the natural skin flora of the human. In other instances the technic employed has been more or less tedious and complicated, seemingly practical only in certain conditions where the time element has not been an important factor.

The ideal germicide for skin disinfection should be effective in causing practically complete destruction of not only surface skin bacteria but also those situated in the deeper layers. Antiseptic action should be maintained for several hours after application. In addition, the ideal germicide should not injure the most sensitive skin, and may best fulfill its function if it directly stimulates tissue cell growth and healing.

Merthiolate (sodium ethyl mercuri thiosalicylate), an organic mercurial compound, seems to fulfill the requirements of a satisfactory disinfectant for the skin. This compound has been shown to possess active germicidal properties, maintaining its effectiveness in the presence of media most nearly resembling the tissues, such as serum agar and white clot or fibrin agar.[9] It is readily soluble, possesses definite penetration properties, and does not precipitate serum proteins. Merthiolate has a low degree of toxicity for animals and human beings, does not hemolyze red blood cells, and does not injure sensitive bacterial antigens and antibodies.[9, 10, 11] It has been found to stimulate tissue cell growth and healing.[12] Merthiolate has also been reported to approach the ideal germicide very closely in tests utilizing bacteria and living tissue growing in vitro.[13] In these new tests, which give most important information as to the real value of a germicide, combining as they do readings on the germicidal value and effect on living tissue, merthiolate has a rating of 0.9, and phenol a rating of 0.2, as against a minimum rating of 1.0 for a theoretically ideal germicide. The close approach of merthiolate to the ideal in these tests indicates that a high degree of efficacy should be realized in tissue antisepsis.

Reimann[14] has reported that some individuals display a sensitiveness to thio compounds, which is characterized by reddening of the treated area and the ap-

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pearance of small papules and vesicles. Since merthiolate is a derivative of a thio compound, the occasional erythema noted in a few highly sensitive persons following the use of unstabilized aqueous merthiolate solution may in part be attributable to this factor. Studies on procedures which bring about stability of solutions and overcome this possible tendency as well as the possibility of infrequent mercurial dermatitis in particularly sensitive persons will be published separately. These studies, which are to be reported soon, show that by rather simple stabilization methods applied to the solvent, merthiolate solution can be so prepared that it may be applied in 1:1000 dilution as wet dressings for twenty-four hours or longer without causing irritation in highly sensitive individuals who previously reacted after one minute exposure to the unmodified solution. Such stabilized merthiolate solutions[12] retain their healing properties. The merthiolate solutions used in the work reported in this paper included stabilized solutions which cause no irritation in either ordinary or hypersensitive subjects.

The work reported here consists of experiments indicating the comparative potency of merthiolate in skin disinfection procedures under conditions approaching as nearly as possible those encountered in ordinary clinical practice. A simple, rapid technic was employed. The organisms dealt with consisted in all cases of those normally resident upon or within the human skin. These included gram-positive cocci and various mixtures and proportions of gram-positive and gram-negative bacilli. Tests were carried out in such a way as to determine the value of merthiolate not only in the preoperative preparation of the human skin, but also in first aid antisepsis. Tincture of iodine U.S.P. was used as a control germicide so that results would be comparative. The merthiolate preparations used included both colored and uncolored 1:1000 aqueous solutions, and colored and uncolored 1:1000 alcohol acetone aqueous solutions.

EXPERIMENTAL

General.—The technic used in this work unless otherwise stated is as follows: Human skin areas of about four inches in diameter were treated with soap and water and then shaved. The skin was next thoroughly scrubbed with tincture of green soap and water by means of a gauze sponge. The area was then rinsed with sterile water and dried with a sterile sponge. At this point a culture was taken from the center of the treated area by rubbing the skin vigorously with a sterile swab wet with broth. This swab was rubbed up in a tube of 10 c.c. of fresh veal infusion broth. Following this a gauze sponge wet with the germicide was rubbed evenly over the area for ten to fifteen seconds. After a one minute exposure the excess of germicide was rinsed off with sterile water and the area quickly dried with a fresh sterile gauze sponge. Bilaterally symmetrical skin areas were used, one germicide being placed on one side and the other on the opposite side. Immediately after a one minute exposure a surface culture was taken by vigorously rubbing a portion of the treated area with a sterile swab wet with broth. This swab was rubbed up in a tube of 10 c.c. of fresh veal infusion broth. Deep skin cultures taken at the same time as the surface skin cultures were made as follows: An area of skin about one-eighth inch by one-half inch was scraped with a sterile scalpel until capillary bleeding occurred.

These scrapings were planted to broth tubes. In addition, immediate subcultures to blood agar plates were made from both the surface and the deep skin cultures, using 1 c.c. amounts. After twenty-four hours' incubation at 37° C. both the surface and deep skin cultures were subcultured in 1 c.c. amounts to fresh 10 c.c. broth tubes. All cultures were read after a further incubation of forty-eight hours, i.e., three days after the tests, and may be listed for convenience as follows:

1. Control culture made after cleansing the skin but before application of the germicide.
2. Surface skin broth culture made one minute after application of the germicide.
3. Subcultures to blood agar plates made from No. 2 within one-half hour.
4. Subcultures to broth tubes made from No. 2 after twenty-four hours' incubation.
5. Deep skin broth cultures made one minute after application of the germicide.
6. Subcultures to blood agar plates made from No. 5 within one-half hour.
7. Subcultures to broth tubes made from No. 5 after twenty-four hours' incubation.

Subcultures were made as indicated above to rule out the possibility of inhibition from an excess of germicide being carried over into the cultures and interfering with the determination of the actual killing of the bacteria. This possibility had been considerably minimized by the rinsing of germicide treated skin areas previously described. The regions selected were distributed quite evenly over the body and included those areas frequently subjected to surgery. Twelve white and three colored individuals offered themselves as subjects for these studies. The tests were carried out during the months of May, June, and July, and various degrees of skin hygiene were encountered.

TABLE I

SUMMARY OF COMPARATIVE SURGICAL SKIN DISINFECTION TESTS WITH MERTHIOLATE, AQUEOUS 1:1000

GERMICIDE	NO. OF SKIN TREATMENTS AND CULTURES	SURFACE SKIN CULTURES			DEEP SKIN CULTURES		
		STERILE	CONTAMINATED	PER CENT STERILE	STERILE	CONTAMINATED	PER CENT STERILE
Merthiolate, aqueous 1:1000	47	47	0	100	43	4	91.4
Control cultures before application of Merthiolate	47	0	47	0			
Tincture of iodine, U.S.P.	40	34	6	85	33	7	82.5
Control cultures before application of tincture of iodine	40	0	40	0			
Distilled Water Control	10	0	10	0	0	10	0

Presurgical Skin Disinfection Tests with Merthiolate, Aqueous 1:1000.—Table I gives a summary of the skin disinfection results obtained with merthiolate, aqueous solutions 1:1000. Because of preliminary washing with soap and water, the aqueous solutions wet such areas adequately. Clear aqueous solutions of merthiolate 1:1000 as well as aqueous solutions colored with various dyes were equally effective, and this agrees with our test tube experiments not reported here. Of 47 merthiolate applications, the surface skin was sterilized in all cases (100 per cent), and the deep skin was sterilized in 43 tests (91.4 per cent). Of 40 tincture of iodine applications under exactly comparable conditions the surface skin was sterilized in 34 cases (85 per cent), and the deep skin was sterilized in 33 tests (82.5 per cent). In 7 of these tests, control tincture of iodine applications were not made, due to the delicate nature of the regions selected. Control tests consisting of washing of similarly prepared areas with sterile water for one minute instead of germicide, resulted in entire failure to sterilize the skin. In all instances the control cultures taken after scrubbing with soap and prior to application of the germicidal agents were positive, showing that the scrubbing was not effective as regards skin disinfection.

TABLE II

SUMMARY OF COMPARATIVE SURGICAL SKIN DISINFECTION TESTS WITH MERTHIOLATE, ALCOHOL ACETONE AQUEOUS 1:1000

GERMICIDE	NO. OF SKIN TREATMENTS AND CULTURES	SURFACE SKIN CULTURES			DEEP SKIN CULTURES		
		STERILE	CONTAMINATED	PER CENT STERILE	STERILE	CONTAMINATED	PER CENT STERILE
Merthiolate, alcohol acetone aqueous 1:1000	57	55	2	96.4	52	5	91.2
Control culture before application of Merthiolate	57	0	57	0			
Merthiolate, alcohol acetone aqueous diluent control	43	19	24	44.1	8	35	18.6
Tincture iodine, U.S.P.	43	43	0	100.0	36	8	83.7
Control culture before application of tincture of iodine	43	0	43	0			

Presurgical Skin Disinfection Tests with Merthiolate, Alcohol Acetone Aqueous 1:1000.—Table II gives a summary of the skin disinfection results obtained with merthiolate, alcohol acetone aqueous 1:1000. In addition to the tincture of iodine control there was also included an alcohol acetone aqueous control, using the same formula for the mixture as was used in the merthiolate, alcohol acetone aqueous solutions. In order to delineate clearly the areas treated, the merthiolate solutions were colored with various dye substances which previous test tube experiments had shown did not modify the germicidal value of merthiolate. One of these solutions, colored with eosin and sodium fluorescein, proved very satisfactory of application, changing in color from yellow to pink upon dry-

ing. Of 57 merthiolate, alcohol acetone aqueous applications the surface skin was sterilized in 55 cases (96.4 per cent), and the deep skin was sterilized in 52 tests (91.2 per cent). Of 43 tincture of iodine applications the surface skin was sterilized in all cases (100 per cent), and the deep skin was sterilized in 36 tests (83.7 per cent). Of 43 control applications using the alcohol acetone aqueous diluent of merthiolate the surface skin was sterilized in 19 tests (44.1 per cent), and the deep skin was sterilized in 8 tests (16.6 per cent). In all cases the control cultures taken after scrubbing with soap and prior to application of the germicides were positive.

Maintenance of Skin Antisepsis with Merthiolate.—In the tests reported above, and in fact in nearly all work heretofore reported, the immediate condition of the skin, i.e., whether sterile or contaminated, has been the only question involved. It seemed desirable to inquire into the duration of antisepsis inasmuch as a long period of antibacterial action is often desirable, especially in cases involving trauma with devitalization of the tissues and in certain operative procedures.

In order to determine the value of merthiolate in this connection, two bilaterally symmetrical abdominal skin areas were prepared, as described previously, in each of ten adult subjects. One area was treated with merthiolate,

TABLE III

COMPARATIVE MAINTENANCE OF SKIN ANTISEPSIS FOLLOWING USE OF MERTHIOLATE

GERMICIDE	NO. OF TEST SUBJECTS	SKIN TESTS MADE AFTER	NO. STERILE	NO. CONTAMINATED	COMPUTED NUMBER SKIN BACTERIA PLANTED IN TUBES PROVING SKIN CONTAMINATED	
Merthiolate, alcohol acetone aqueous 1:1000	10	15 min.	9	1	40	
	10	1 hour	8	2	20;250	
	10	2 hours	8	2	10;200	Total 760
	10	3 hours	8	2	10; 80	
	10	5 hours	9	1	150	
Tincture iodine, U.S.P.	10	15 min.	9	1	500	
	10	1 hour	10	0	0	
	10	2 hours	9	1	200	Total 790
	10	3 hours	9	1	20	
	10	5 hours	8	2	40;30	

alcohol acetone aqueous 1:1000, and the corresponding area with tincture of iodine. Surface skin cultures and deep skin cultures were taken at the end of five minutes and appropriate subcultures were made. A sterile gauze dressing was then applied to each area and the subjects went about their usual work. Culturing of each treated area was repeated after one, two, three, and five hours. During the time of the tests the temperature was above 90° F. and some of the subjects perspired freely. The results of these tests are summarized in Table III. They indicate that both merthiolate and tincture of iodine maintain relatively good antiseptic action under rather severe conditions. Those cultures taken after application of the germicide which proved positive originated from relatively few bacteria as compared to the enormous numbers of bacteria recovered from untreated skin. Two of the ten subjects used in this experiment

showed toward the close of the five-hour test period quite intense erythema in the areas treated with tincture of iodine. The merthiolate, alcohol acetone aqueous solution did not provoke any skin reaction. It was regularly noted that the abrasions made in merthiolate (aqueous or alcohol acetone aqueous) treated areas for deep skin cultures healed more rapidly than similar abrasions in the iodine treated areas. This phase of the work is the subject of a separate report.[12]

Merthiolate in First Aid Skin Disinfection Procedures.—Merthiolate, alcohol acetone aqueous 1:1000 and tincture of iodine, U.S.P. have been used in a number of tests upon the untreated skin of individuals, utilizing areas such as the dorsum of the hand and foot, shin, forearm, and upper arm. In these subjects previous washing with soap and water was omitted, and the tests did not involve trauma. It has been regularly found that the skin treated with either merthiolate or tincture of iodine is sterilized in a high proportion of cases. In those where contamination remained, the number of surviving organisms was infinitely small compared to those present in the normal skin. These results are in accord with those listed in detail above, showing the efficiency of merthiolate as a skin disinfecting agent.

SUMMARY

1. The effectiveness of merthiolate (aqueous and alcohol acetone aqueous) in preoperative and first aid skin disinfection is reported.

2. Artificial test conditions were avoided by:

a. The use of the natural flora of the skin as test organisms.

b. The employment of a technic as nearly similar as possible to that used in regular clinical practice.

3. The tests indicate that merthiolate approaches the ideal germicide for skin disinfection procedures because of the following properties demonstrated under conditions comparable to actual clinical use:

a. High germicidal activity against surface skin organisms.

b. High germicidal activity against deep skin organisms.

c. Rapidity of skin disinfection.

d. Maintenance of condition of antisepsis over a considerable period of time.

e. Nonirritating to the most sensitive skin.

f. Freedom from vapors irritating to the eyes of the operators and attendants.

g. Promotion of healing of abrasions by actively stimulating tissue cell repair.

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REFERENCES

1. Reddish, G. F., and Drake, W. E.: Mercurochrome 220 Soluble and U.S.P. Tincture of Iodine, J. A. M. A. **91**: 712-716, 1928.
2. Scott, W. W., Hill, J. H., and Ellis, M. G.: Action of Mercurochrome and Tincture of Iodine in Skin Disinfection, J. A. M. A. **92**: 111-116, 1929.
3. Raiziss, G. W., Severac, M., and Moetsch, J. C.: Metaphen as a Germicide and Skin Disinfectant, J. A. M. A. **94**: 1199-1201, 1930.
4. Scott, W. W., and Birkhaug, K. E.: Comparative Value of Metaphen in Alcohol-Acetone-Aqueous Solutions in the Preoperative Disinfection of the Skin, Ann. Surg. **93**: 587-597, 1931.

5. McKenna, W. F., and Fisher, H. A.: The Use of Potassium-Mercuric-Iodide for Skin Disinfection, Surg. Gynec. Obst. **30**: 370-373, 1920.
6. Tinker, M. B., and Sutton, H. B.: Inefficiency of Most of the Commonly Used Skin Antiseptics, J. A. M. A. **87**: 1347-1351, 1926.
7. Tinker, M. B., and Sutton, H. B.: Skin Disinfection, With Especial Reference to the Use of Acriflavine, J. A. M. A. **88**: 1560-1561, 1927.
8. Bonney, Victor, and Browning, C. H.: Sterilization of the Skin and Other Surfaces by a Mixture of Crystal Violet and Brilliant Green, Brit. M. J. **1**: 562-563, 1918.
9. Powell, H. M., and Jamieson, W. A.: Merthiolate as a Germicide, Am. J. Hyg. **13**: 296-310, 1931.
10. Jamieson, W. A., and Powell, H. M.: Merthiolate as a Preservative for Biological Products, Am. J. Hyg. **14**: 218-224, 1931.
11. Marshall, M. S.: Merthiolate—A New Antiseptic, California & West. Med. **35**: 43-44, 1931.
12. Powell, H. M., Jamieson, W. A., and Kempf, G. F.: The Healing Properties of Merthiolate, Am. J. Hyg. **15**: 292-297, 1932.
13. Buchsbaum, R., and Bloom, Wm.: Relative Toxicity of Antiseptics on Bacteria and Tissues in Cultures, Proc. Soc. Exper. Biol. & Med. **28**: 1060-1064, 1931.
14. Reimann, S. P.: Thiocresol in Wound Healing, Ann. Surg. **93**: 624-627, 1931.

TREATMENT OF RHEUMATIC FEVER WITH A MAGNESIUM CINCHOPHEN, MAGNESIUM OXIDE (MAGNEPHEN) PREPARATION*

By Edward Tolstoi, M.D., and Doreen R. Corke, M.D., New York City

THE medicinal treatment of rheumatic fever has been limited chiefly to salicylates and combinations of the phenylcinchoninic acid group. These drugs in no way alter the course or duration of an attack; they do, however, permit a more rapid convalescence by alleviating the joint pains, lowering the temperature, and also possibly by eliminating the intoxication.[1] It is now well established that even though the temperature approaches the normal level and there are no joint pains, activity of the rheumatic virus may be present under most adequate medication, and evidences of such activity are leucocytosis, increased sedimentation time of erythrocytes and even the development of cardiac lesions.[1, 2] While most of the time salicylates and the various phenylcinchoninic preparations make the patient comfortable by relief of pain, the intoxication caused by these drugs offsets at times the advantages obtained from their use. The buzzing in the ears, headache, nausea, vomiting, and sometimes the diarrhea are most annoying and such symptoms may be produced even by small doses, depending on the sensitivity of the individual to the drug used.

To combat this disadvantage, Lees[3] suggested the administration of sodium bicarbonate with salicylates. He believed that their toxicity was reduced by the combination. This assertion, however, has not been substantiated by others. It has been the usual practice to use substitutes when evidences of salicylate intoxication were apparent. Unfortunately such substitutes were also without therapeutic value unless given in large doses which, too, were toxic. Hanzlik[4] believes that the search for substitutes or derivatives of these drugs is futile. He is of the opinion that a better tasting and perhaps less soluble and less irritating combination may be evolved, but its effect may not be so marked as is noted by the use of the salicylates or cinchophen preparations. "Full therapeutic efficiency and toxicity go hand in hand" and—"absence of toxicity means absence of therapeutic efficiency," states Hanzlik.

While searching for a combination which would be less toxic and still have its full therapeutic effect, Barbour and Winter[5] have found that magnesium salts potentiate the activity of salicylates, and members of the phenylcinchoninic acid group. These investigators found evidence that the magnesium salt of phenylcinchoninic acid is less toxic for mice than the sodium salt. They also observed that the magnesium compound was a more efficient antipyretic in fevered rabbits, and furthermore the addition of magnesium chloride to the magnesium com-

*From the Medical Service, Beekman Street Hospital.
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These tablets are known commercially as "magnephen" and were supplied to us through the courtesy of the Calco Chemical Company.

pound of phenylcinchoninic acid produced more marked onset of antipyresis and they expressed the view that it pointed to a temporary synergism of the compound.

With such advantages claimed for a preparation, we were anxious to use it in the treatment of cases of rheumatic fever which were under our care. The magnesium compound of the magnesium salt of phenylcinchoninic acid was used. This was made up in tablets of 7½ grains (0.5 gm.) each, thus making its administration simple and enabling us to keep accurate records of the amount used. Each tablet contained 4 grains (.27 gm.) of magnesium cinchophen and 3 grains (.20 gm.) grains of magnesium oxide. One hundred grains (6.6 gm.) of the new preparation are actually equivalent to 45 grains (3.0 gm.) of phenylcinchoninic acid. Our series of cases is not very large. The observations, however, have been careful, the data complete, and the results so promising and uniform that we felt justified in presenting these cases so that others may be encouraged to use these and other similar preparations.

There has been a tendency recently to avoid the use of cinchophen and its various combinations, because of its believed toxic action on the liver. Numerous publications both in this country and abroad have shown that a hepatitis and occasionally acute yellow atrophy of the liver result from the use of this drug. Barron[6] has shown that even 15 grains (1.0 gm.) can bring on an attack of shock within two hours. His patient recovered. Evans[7] reported three cases of hepatitis following the use of such preparations. Among the other numerous reports in the more recent ones of Sutton[8] and Rabinowitz,[9] Parsons and Harding,[10] and Sherwood and Sherwood,[11] more evidence is adduced both from personal observations as well as complete reviews of the literature, as to the toxicity of these preparations and particularly their predilection for liver damage, often with fatal results. Churchill and Van Wagoner[12] produced cinchophen poisoning in dogs by using 27 times the therapeutic dose. These large doses produced acute gastric ulcers, and some liver damage, as was shown by the diminished liver function. Six of their animals died. When, however, the therapeutic dose of cinchophen was fed, even though the kidneys were artificially damaged by clamping the renal artery, no death resulted, but only slight transient manifestations of liver damage. Fishberg,[13] fed therapeutic and even toxic doses of the drug to rabbits and was unable to find signs of liver damage either grossly or microscopically. He also administered a soluble cinchophen preparation to rabbits by vein and could not demonstrate pathologic changes in the liver. With reports that are not quite unanimous and knowing that the phenylcinchoninic group preparations have been and are still used extensively, we felt that their damaging effect is far from constant. It may be that certain individuals are sensitive to them, and it is in such cases that the hepatitis results. In other words, it is not the drug but the patient who is at fault. The knowledge, however, that untoward results may be brought about by the use of the cinchophen group, warrants most careful vigilance, and the earliest evidences of toxicity, such as anorexia, vomiting, urticaria or jaundice, dictate their immediate discontinuance. We have used a cinchophen compound in the treatment of our cases, fully cognizant of the possible hazards and consequently most careful in our

observations and care. We insisted that our patients have ample carbohydrate in their diet. This we felt afforded some protection to the liver.

Even though we have treated seven cases of rheumatic fever, we are only reporting four in detail. The other three were treated when we were not very familiar with the drug and we felt unjustified in subjecting acutely ill patients to experiment when an established remedy was at hand. With the earlier cases, therefore, we were cautious in the administration of the new preparation as we knew nothing of the dosage or its by-effects on human beings. We administered large doses of salicylates to the patients when they were acutely ill and after their symptoms were considerably relieved we substituted the magnesium phenylcinchoninic acid preparation. At first we used 7½ grains (0.5 gm.) every four hours. Since no toxic effects were noted, we gradually increased the dosage to as high as 30 grains (2.0 gm.) every four hours. In our limited experience 75 grains (5.0 gm.) to 90 grains (6.0 gm.) per day were ample to produce therapeutic effects without concomitant toxicity. As we learned more about the preparation, we used it as soon as the cases were admitted, and the four cases we are presenting are the results of such observations. The cases here reported were in the acute phase of the disease. On admission each patient received the routine care and, after a physical examination, blood examination for its count, sedimentation time and the ictertic index of the serum, the medication was started. The total dosage was distributed throughout the day at four-hour intervals. Our patients were kept on medication and in bed until all evidences of activity were gone. The temperature and pain were the first to respond. The patients then felt well and asked for certain liberties, stating that they were well enough to be up. Their requests were rejected as long as there was a leucocytosis and an increased sedimentation time. When, however, the temperature dropped, the pain vanished, and the latter two criteria approached normal limits, the medication was discontinued, the patient was allowed out of bed by degrees, and after a week or ten days of further observation without any medication, discharged.

CASE 1.—(No. 1563.) A male Chinese laundryman, aged thirty-three, was admitted March 3, 1931. He complained of pains in the left and right hips, knees, ankles, shoulders, and wrists for four days previous to admission; the pains were accompanied by dyspnea and cough, and a purpuric rash appeared on both legs. No history of previous attacks was obtained and there were no contributory facts, either in his past or family history. Temperature 102°, respiration 22, pulse 136. He appeared acutely ill, was moderately dyspneic and slightly cyanosed. The teeth and gums were in poor condition, the throat injected, and the tonsils inflamed. The heart showed a diffuse heaving impulse and appeared enlarged to percussion. The sounds were forceful, totally irregular and rapid. In the pulmonic area a rough systolic murmur was heard. Blood pressure 110/66. The liver edge was felt below the costal arch; the spleen was not felt. Over both legs there were numerous ecchymotic areas, varying in size from that of a pinhead to that of a large pea. There was tenderness in the joints but no swelling or redness. The blood Wassermann and blood cultures were negative. Coagulation time seven minutes, bleeding time two minutes, clot retraction normal. R.B.C. 4,660,000, H.B.O. 70 per cent, W.B.C. 6,800, with a practically normal differential count. Sedimentation time was 30 per cent. The electrocardiogram showed auricular fibrillation; poor electromotive force and small upright T-waves. The patient was immediately placed on "magnephen," 75 grains (5.0 gm.) daily. The pain and tenderness disappeared within three days, and incidentally, the purpuric rash began to fade. The temperature dropped to normal on the fourth

day and the heart rate was 90. With the slower heart rate, evidences of mitral stenosis were made out. For seven days he was comfortable. He then developed a red throat and an abscess of the gum above the upper left second bicuspid. This was incised, following which the temperature dropped to normal, remaining at this level until discharged. March 28, twenty-five days after admission, medication was discontinued as the temperature had been normal for ten days and the leucocyte count was 7,000 with the sedimentation time of 5 per cent, and on discharge, April 9, it was again 5 per cent. This patient had ingested 1,875 grains (125.0 gm.) of the preparation and at no time were there symptoms of toxicity and the icteric index on discharge was 5.

CASE 2.—(No. 15611.) An Italian boy thirteen years old was admitted on February 27, 1931, complaining of pains in the knees, ankles, wrists, and small joints of the hands for ten days. The present attack was initiated by a sore throat and cold in the head. There was a history of a previous attack of rheumatic fever three years before and the patient was under observation in the out-patient department. Other than a systolic murmur on his former admission, no abnormalities of the heart were recorded. He had had measles in early childhood and a tonsillectomy when three years old. The physical examination revealed a well-nourished and well-developed pale boy. The previously observed systolic blow at the cardiac apex was noted, the liver was just palpable and there were swelling and tenderness in both ankles and the right third metacarpophalangeal joint. On admission white count was 12,500 with 72 per cent polys. The Wassermann was negative and the urinalysis revealed a trace of albumin. He was treated with sodium salicylate, 90 grains daily, as his temperature was 104° and he was obviously in the acute phase of the disease. We preferred salicylates at first here, since we were eager to bring about symptomatic relief rapidly and did not feel justified in experimenting with a drug about which we knew little. On the third day he was practically symptom-free, on the fifth day the temperature dropped to normal and on the seventh day medication was stopped. About a week after medication was discontinued the W.B.C. were 14,750 and three days later the temperature rose above 100°, being accompanied by pain in the small joints. At this time the magnesium preparation 75 grains (5.0 gm.) daily was used instead of salicylates. The sedimentation time was 50 per cent. Following the administration of the drug subjective improvement was noted almost immediately. The temperature oscillated between 98.2° and 100.6° for eighteen days in spite of the medication. The leucocyte count was dropping, being 11,300 on April 7, and the sedimentation test was 25 per cent at about the same time. The amount of the drug was then increased to 90 grains (6.0 gm.) daily, causing a drop in temperature to normal and after ten days of normal temperature a leucocyte count of 9,900 and a sedimentation time of 11 per cent, the patient was allowed out of bed and discharged a few days later. No nausea, ringing in the ears, jaundice, or urticaria were noted and the icteric index on discharge was 5 units. The patient had ingested 1,425 grains (95.0 gm.) of magnephen.

CASE 3.—(T. V.) A white man fifty years old, a painter, was admitted on May 2, 1931, because of fever and pain and swelling in the wrists, ankles, elbow joints, and knees. No redness was noted by the patient. These symptoms were present for a short time before admission. He stated that during the past twenty years he had had attacks of "rheumatism" and three years ago had had a tonsillectomy. Venereal infection was denied.

The admission temperature was 101°. The patient, who was well nourished, was in pain and could not move his hands or feet. The teeth were in poor condition, and small tonsillar tags were present. The heart and lungs revealed no abnormalities; blood pressure 114/72. There were swelling and tenderness about the right wrist, elbow, and left ankle. No abnormalities were noted in the red blood cells by count or morphology on four studies. The admission W.B.C. were 6,800 and at no time was there a rise noted. The sedimentation time was 45 per cent on admission, the respective weekly determinations subsequently were 18 per cent, 10 per cent and 4 per cent. The blood Wassermann was negative and the icteric index 4 units both at the beginning and at the end of the treatment. This patient was given 75 grains (5.0 gm.) magnesium phenylcinchoninic acid preparation daily, in 15 grain doses. As his pains were quite severe, he was given codeine ½ gr. every four hours for the first twelve

hours. Within twenty-four hours the temperature became normal and remained so for the rest of his hospital stay. The swelling and tenderness disappeared from the joints involved, by the third day. He frequently complained, however, of pain in the ankles, knees, wrists, and elbows. No swelling or tenderness accompanied these pains. The magnephen was then increased to 90 grains (6.0 gm.) on May 14, daily, with the consequent relief of pain. The drug was discontinued on May 28, 1931. This patient received a total of 2,130 grains (142 gm.) and at no time were toxic symptoms observed. After he was free of symptoms tonsillar tags were removed and he was then discharged on June 15, 1931.

CASE 4.—(Hospt. No. 15810.) On March 25, 1931, a thirty-eight-year-old Italian was admitted because of pain in the right knee and shoulder. He had been ill for three days and the above symptoms were accompanied by a sore throat, loss of appetite, and fever. The physical examination revealed a well-nourished male whose teeth were in poor condition, the throat congested, the tonsils small, red and cryptic. The heart and lungs presented no abnormalities. The right knee and shoulder joints were tender and hot. There was considerable limitation of motion, but no swelling. The red blood cells revealed no abnormalities. The white count was 15,600 with a 73 per cent poly count. The blood Wassermann was negative. Blood sugar 80, nonprotein nitrogen 40. The icteric index was 4 and the sedimentation time 50 per cent. The urine revealed nothing abnormal. On admission in the acute phase with severe joint pains and a temperature of 103°, he was placed on 75 grains (5.0 gm.) of magnephen in 15 grain doses. Symptomatic relief was noted in three days and the temperature was normal in five. The sedimentation time, however, was still high, 25 per cent. The medication was, therefore, continued for eighteen days in spite of the normal temperature, until both the leucocyte count and sedimentation time became normal, 8,200 and 5 per cent, respectively. The medication was then discontinued and he was allowed out of bed. No toxic symptoms were noted at any time, even though the patient had taken 1,575 grains (105 gm.) of the medication.

SUMMARY AND CONCLUSIONS

From data of the case reports it is obvious that the magnesium cinchophen with the added magnesium oxide fulfills certain desiderata; namely, antipyresis, analgesia and freedom from toxic symptoms, even when administered in therapeutic doses. The magnesium apparently intensifies the phenylcinchoninic acid effect, thus making smaller dosage adequate and consequently the medication need not be pushed to a point of toxicity before symptomatic relief is obtained. It is also apparent that this preparation does not cause cessation of activity as the leucocytosis and elevated sedimentation time persist, in spite of the alleviation of symptoms. We have given the magnesium phenylcinchoninic acid (magnephen) compound in large doses. The first patient received 1,875 grains (125.0 gm.) over a period of 25 days; the second case had 1,425 grains (95.0 gm.); the third 2,130 grains (142.0 gm.) over 26 days; and the fourth 1,575 grains (105.0 gm.) over a period of about 25 days. In none of these were there evidences of drug toxicity. We feel that these results are sufficiently encouraging to warrant further careful study of the magnesium phenylcinchoninic acid compound which promises to do all that is claimed for salicylates and cinchophen in the treatment of rheumatic fever, without producing any disagreeable or toxic symptoms. We have not studied the effect of this preparation on the kidney. The urines of our patients on discharge revealed no abnormalities to cause further tests of renal function. We have also kept in mind the reports of the occasional harmful effect of phenylcinchoninic acid preparations on the liver. The patients were observed for evidences of jaundice and the icteric index was

done on admission and discharge. No evidence of liver injury was noted clinically, and the icteric index showed no change after the ingestion of considerable quantities of the drug.

From our clinical study of seven cases of rheumatic fever, four of which we have discussed in detail, we offer the following conclusions:

1. Magnesium oxide in combination with magnesium cinchophen (magnephen) is an effective antipyretic and analgesic in the treatment of rheumatic fever.

2. The therapeutic dose, because of the magnesium potentiation, is much smaller and does not produce toxic symptoms.

3. Large doses such as 2,130 grains (142 gm.) over twenty-six days were well tolerated.

4. This preparation does not influence the activity of the rheumatic virus as is evidenced by a persisting leucocytosis and an increased sedimentation time. It produces symptomatic relief by reducing the temperature and relieving the arthralgia.

5. The advantage of the preparation is the absence of toxicity, when administered in therapeutic amounts. This has been our experience with the cases studied.

REFERENCES

1. Swift, Homer F.: The Treatment of Rheumatic Fever, Boston M. & S. J. **187**: 331, 1922.
2. Ernstene, Carlton A.: Erythrocyte Sedimentation, Plasma Fibrinogen and Leucocytosis as Indices of Rheumatic Infection, Am. J. M. Sc. **180**: 12, 1930.
3. Lees, D. B.: The Effective Treatment of Acute and Subacute Rheumatisms, Proc. Roy. Soc. Med. p. 2-34, 1908, 1909.
4. Hanzlik, P. J.: Salicylates and Cinchophen in Medicine, Medicine **5**: 197, 1926.
5. Barbour, H. G., and Winter, J. E.: Antipyretic Action and Toxicity of Combinations of Magnesium With Phenyl Cinchoninic Acid, J. Pharm. & Exper. Therap. **35**: 425, 1929.
6. Barron, Moses: Cinchophen Poisoning, J. A. M. A. **82**: 2010, 1924.
7. Evans, Geoffrey: Atophan Derivatives in Rheumatism, Brit. M. J. **2**: 93, 1926.
8. Sutton, Don C.: Acute Yellow Atrophy of the Liver Following the Taking of Cinchophen, J. A. M. A. **91**: 31, 1928.
9. Rabinowitz, Meyer A.: Atrophy of the Liver Due to Cinchophen Preparations, J. A. M. A. **95**: 1229, 1930.
10. Parsons, Lawrence, and Harding, Warren G. 2nd: Cinchophen (Atophan) Poisoning, Am. J. M. Sc. **181**: 115, 1931.
11. Sherwood, K. K., and Sherwood, H. H.: Acute Toxic Hepatitis, Arch. Int. Med. **1**: 48, 1931.
12. Churchill, T. P., and Van Wagoner, F. H.: Cinchophen Poisoning, Proc. Soc. Exper. Biol. & Med. **28**: 581, 1931.
13. Fishberg, A. M.: Personal Communications.

THE PREDIABETIC STATE: ITS TREATMENT BY THE LOW CARBOHYDRATE DIET AND THE REDUCTION OF WEIGHT*

By James D. Tyner, M.D., Clifton Springs, N. Y.

MANY writers recognize in the obese an incipient impairment of carbohydrate metabolism which is known as the prediabetic state.[1] The predisposition of the obese to diabetes mellitus is a generally accepted fact.[2] Numerous cases of diabetes must have had their beginning in a state of obesity with a similar impairment of function. The question of proper prophylaxis for this group, against diabetes, logically arises.

This should consist, theoretically, of a reduction in weight and a low carbohydrate diet so that the metabolic apparatus may recover its function. The amount of body tissue to be maintained will thus be lessened and the ingested carbohydrate to be metabolized will be lowered. Whether or not this therapy is successful can best be determined by repeated tests of the carbohydrate tolerance.

The Brill test meal of 100 grams of carbohydrate, 26 grams of protein and 27 grams of fat is used as a test of the carbohydrate tolerance in this clinic.[3, 4, 5] It is given as a breakfast in the fasting state. A blood sugar is taken by venipuncture immediately before and again two hours following the ingestion of the meal. The night urine is collected one hour before the test, while that for the four hours following makes up the second specimen. The blood sugar values are determined by the Benedict copper method; the normal reading of which is 70 to 90 mg. per 100 c.c. of blood.[6] The test is considered normal if a blood sugar of 100 mg. or less is attained in the second blood specimen taken two hours after the meal.

In Table I ten cases of obesity with an impaired tolerance are presented; hence prediabetics, as determined by the above method. All cases have been thoroughly studied in this clinic and none recorded in which were found factors that were known to affect the test. The heights and weights were determined minus clothing and without shoes. From these, and a normal standard,[7] the percentage overweight was calculated. The age given in each case is that at the first tolerance test. After a varying period on a restricted, low carbohydrate diet during which weight was lost, the meal was repeated and the improved tolerance found as tabulated in each second test of Cases 1 through 10.

The rationale of the treatment is further justified by consideration of Cases 11 and 12 (Table I) both of which demonstrate the loss of tolerance for carbohydrate through lack of diet and increasing obesity.

It would appear that the carbohydrate tolerance of the prediabetic can be improved by the reduction of weight and proper diet and that it depreciates with

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gain in weight and dietary indiscretions. The relative importance of the low carbohydrate diet and the reduction of weight needs no discussion. Observance of the first, obviates the second and, to this writer, is paramount.

These facts should be brought to the attention of the obese and prediabetic. It is only by such prophylaxis that a large group may be saved from future diabetes mellitus.

TABLE I

CASE	DATE	SEX	AGE	HEIGHT		WEIGHT		BLOOD SUGAR MG. 100 C.C.		% URINE SUGAR		DIET		
				FT.	IN.	LBS.	% ABOVE OR BELOW NORMAL	FASTING	2 HR.	FASTING	4 HR.	C	P	F
1	12/28/27	F.	55	5	3	178	+20	118	115	0	0	102	75	90
	12/27/30					168	+13	85	100	0	0			
2	10/16/27	F.	50	5	5	170	+12	97	100	0	0	1200 cal.		
	3/24/31					145	+3	70	77	0	0			
3	8/11/28	F.	38	5	6	181	+20	88	107	0	0	108	75	60
	8/11/30					155	0	75	93	0	0			
4	10/11/26	M.	56	5	7	184	+10	89	132	0	0	126	90	204
	9/12/28					171	+4	82	110	0	0			
5	9/25/28	F.	27	5	0	131	+4	96	170	0	0	135	90	54
	5/ 2/29					115	−9	75	100	0	0			
6	9/15/29	F.	37	5	4	148	+6	95	132	0	0	150	60	75
	10/ 3/29					140	0	97	95	0	0			
7	12/ 6/30	M.	27	5	7	264	+72	95	120	0	0	225	120	190
	2/29/31					224	+53	79	81	0	0			
8	11/30/25	F.	40	5	4	168	+14	100	132	0	0	120	72	60
	7/ 8/27					140	−5	80	83	0	.13%			
9	5/10/30	F.	50	5	4	198	+32	80	110	0	0	120	72	60
	8/15/30					173	+15	70	88	0	0			
10	5/19/28	M.	44	5	7	195	+22	104	137	0	0	135	90	75
	11/29/30					165	+4	72	75	0	0			
11	1/29/30	F.	69	5	4	183	+17	94	83	0	0	No restrictions		
	12/17/30					193	+21	91	111	0	0			
12	10/18/28	M.	45	5	6	190	+26	100	97	0	0	No restrictions		
	9/17/29					210	+35	95	116	0	0			

CONCLUSIONS

1. Repeated carbohydrate tolerance tests on the obese and prediabetic demonstrate that tolerance depreciates with increasing weight and without a low carbohydrate diet, but that it improves with loss of weight and a diet low in carbohydrate.

2. It is suggested that the obese and prediabetic be warned of this danger and advised of the necessity of reduction of weight and a restricted carbohydrate intake.

3. The development of diabetes mellitus in a large group might be prevented by these measures.

REFERENCES

1. Paullin, J. E., and Sauls, H. C.: Study of the Glucose Tolerance Test in the Obese, Southern M. J. **15**: 249, 1922.
2. Joslin, Elliott P.: The Prevention of Diabetes Mellitus, J. A. M. A. **76**: 79, 1921.
3. Brill, I. C.: The Effect of a Normal Meal Upon the Blood Sugar Level in Health and in Certain Conditions of Disease, J. LAB. & CLIN. MED. **8**: 727, 1923.
4. Hubbard, R. S., and Wright, F. R.: Demonstration of Impairment of Carbohydrate Tolerance by Brill's Test Meal, Clifton Med. Bull. **12**: 155, 1926.
5. Allison, Catherine S.: Blood Sugar Values Before and After a Standard Test Meal by Benedict's Copper Reduction Method, Clifton Med. Bull. **14**: 23, 1928.
6: Benedict, S. R.: The Determination of Blood Sugar, J. Biol. Chem. **34**: 203, 1918.
7. Joslin, Elliott P.: Treatment of Diabetes Mellitus, 1923, Lea and Febiger, p. 761.

THE STERILIZATION AND STANDARDIZATION OF PAPAIN PREPARATIONS INTENDED FOR SURGICAL USE*

By Robert P. Walton, Ph.D., New Orleans, La.

PROTEOLYTIC enzymes are being used by Oehsner and Garside[1] and others as a surgical means of preventing the reformation of peritoneal and pericardial adhesions. According to this procedure, adhesions are divided by surgical means, and their reformation prevented by the presence of weak protease solutions. The fibrinous exudate is presumably digested to such extent as to prevent the formation of fibrous tissue. In contrast to trypsin, papain preparations have proved much more suitable in that they are more consistently effective and, further, are effective at spectacularly low activity concentrations.[1] This is apparently due to the markedly greater stability of the vegetable enzyme, papain, in aqueous solution and to its capacity to act uninhibited in the presence of serum. Quantitative comparisons of this sort and comparative rates of deterioration in the peritoneal cavity, as determined by methods described here, are reported in another communication.[2]

This paper describes the sterilization procedure, the storage behavior and the activity standards of papain preparations which are being used for experimental and clinical work.

1. PREPARATION OF A STERILE PRODUCT

In a previous report[3] sterile and stable trypsin preparations were conveniently obtained by two procedures. These have been applied in the case of papain with varying success. The first, which consisted in pressure filtration of a glycerine extract of the powder through Berkefeld filters, was discarded because of the relative instability of the glycerine papain filtrate. The second procedure, which consisted in a similar filtration and subsequent precipitation of the active powder with alcohol and ether under sterile conditions, presented no unexpected difficulties when applied to papain and the stability of the resulting powders was found to be entirely adequate. The firm of Parke Davis and Company have cooperated in developing a product of this latter type intended for clinical use.

Glycerin Papain Filtrates.—In the same way as with trypsin, glycerine extracts of papain filtered by pressure through Berkefeld filters (N) furnished products of uniform activity. Using 5 gm. of powder dissolved or suspended in 100 c.c. of 60 per cent glycerine, the activity of the filtrate is such that about 22 c.c. is equal to one gram of the dry powder.

Numerous assays on 12 different lots over periods up to four months showed that filtrates stored open in the refrigerator averaged a 50 per cent activity-loss

*Department of Pharmacology, School of Medicine, Tulane University.
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in one month, while ampouled filtrates stored in the refrigerator averaged a 50 per cent activity-loss in four months. The rate of deterioration was observed by two methods to be described in Section 2. One is based on the time required to disintegrate approximately equal amounts of glycerine-stored fibrin and the other is expressed as units of activity necessary to dissolve the gelatine on a strip of photographic film.

A typical filtrate stored open in the refrigerator showed initially a "fibrin digestion time" of one and eight-tenths hours, after six days, two and two-tenths hours, after fourteen days, three and one-half hours and after ninety days, eight hours. In each digestion test 0.5 c.c. of the filtrate was used. Using this amount of filtrate, the "fibrin digestion time" for several freshly prepared lots was consistently within the limits of one and one-half to two hours. As these latter tests ranged over a period of four months, it is considered that the digestibility of the glycerine stored fibrin was not much altered.

Using the photographic film method and expressing activity in arbitrarily defined units the same type of storage behavior was noted. Fresh preparations exhibited 45 to 48 activity units per cubic centimeter. Typical assays on filtrates stored open in the refrigerator showed the following unitages per cubic centimeter: nine days, 42 units; nineteen days, 31 units; thirty-five days, 24 units; one hundred and five days, 20 units.

A lesser number of observations (about 10) were obtained with filtrates stored in sealed glass ampoules. The average indicated a slower rate of deterioration than open preparations.

Although considerable variations were observed in the deterioration rate of various specimens, the results were sufficiently consistent to indicate the inadvisability of glycerin filtrates for general clinical use. Because of the convenience of preparation and use, however, this type of product has been useful in laboratory experimentation.

That the activity of trypsin in glycerin solutions is much more constant was indicated in the previous report and has since been supported by further observations. One ampouled specimen, for instance, stored in the refrigerator for eight months exhibited an activity-loss of less than 10 per cent.

No experimental explanation was developed for this lesser stability of papain on storage in glycerin although some unsuccessful attempts were made. Contact with mercury vapor for one month at ice chest temperatures indicated no particular inactivation from this source. Slow streams of oxygen and of carbon dioxide bubbled through the solutions for twelve and fifteen hours at ordinary temperatures did not cause an activity-loss greater than 10 per cent. When poured in shallow dishes and exposed to ultraviolet rays for two hours, the same order of stability was noted. The aqueous glycerin solutions at 60 per cent concentration were shown not to undergo dilution by absorbed moisture under the usual conditions of storage.

Filtered and Precipitated Papain Powders.—According to this procedure, aqueous or 60 per cent glycerine solutions of papain were filtered as before, precipitated with 5 volumes ethyl alcohol, treated successively with anhydrous alcohol and anhydrous ether and allowed to dry in a desiccator containing, sep-

arately, concentrated sulphuric acid and sticks of alkali. Separation of the powder from the suspension liquid is best effected by centrifuging. Ordinarily, 80 to 90 per cent of the filtrate-activity can be recovered in the precipitate. Chloretone or acriflavine can be used as precautionary antiseptics, since they have only a very slight effect on papain activity.

In that these operations required sterile technic throughout, the laboratory preparation was most conveniently effected by placing one to two c.c. quantities of filtrate in ordinary test tubes and carrying out the operations of precipitation, centrifuging and drying in the same tube in which it was ultimately sealed for use. This was possible since these quantities represented as much or more than the usual therapeutic dosage.

Preparations of this sort exhibited the normally expected stability, as determined over periods up to forty-five days. One preparation stored for twenty days at 50° in a sealed ampoule exhibited an activity loss not greater than 10 per cent.

According to the method now used by Parke Davis and Company, aqueous extracts are prepared by overnight contact, filtration and precipitation with methyl alcohol. Other details are much the same as described above. These products are more completely soluble than the untreated powders and the relation of protein-dissolving activity to protein-hydrolyzing activity is distinctly greater than with the untreated powders. These latter relations are given in Section 2. The protein-dissolving activity of these sterile powders, now being used in the laboratory and in the clinic, corresponds roughly, milligram for milligram, to that of the unsterilized stock preparations. This is a matter of convenience in expressing activity as most of the surgical and pharmacologic work done here has been based on either one or the other of these two products.*

With the expectation of simplifying the preparative procedure, observations were made as to the storage behavior of the precipitate when allowed to remain in contact with the precipitating alcohol. The deterioration rate was somewhat greater than that of the glycerin filtrates but of the same general order. One preparation, for instance, kept sealed in the ice chest for thirty-five days, showed a change of "fibrin digestion time" from three to six hours. These observations have an incidental interest in demonstrating that the papain precipitates are not markedly affected by the presence of the precipitating alcohol. Accordingly, time of contact with alcohol is not an important factor in standardizing the preparative procedure.

Sterilization by Carbon Disulphide.—Recently, Vandevelde[4] has reported effective sterilization of enzyme powders by prolonged treatment with freshly distilled CS_2 at ordinary temperatures. Periods of several days were considered adequate, although in an earlier report[5] six weeks of contact were employed in sterilizing flours. No statements are given as to the number and types of bacterial contaminants. From the following observations it is seen that the method, as described, is not suitable for the sterilization of papain powders, when highly contaminated with B. subtilis.

*The activity and solubility of these untreated, stock preparations did not differ essentially from a stock preparation of Mercks "White Label" papain. However, a special papain preparation, kindly supplied by Wallerstein laboratories exhibited a substantially greater activity.

Portions (100 mgm.) of untreated powder from the same lot throughout were weighed into sterile test tubes and covered with about 6 c.c. of CS_2. The tubes, already drawn out, were sealed by flame. After standing for various periods of time, the tubes were broken and the CS_2 allowed to evaporate under the protection of sterile cotton plugs. The powders were dissolved in sterile water and 1/10 of the solution cultured on agar plates. The results are given in Table I.

TABLE I

TIME OF CONTACT	CARBON DISULPHIDE	COLONIES PER 100 MG. NEUTRAL AGAR	MEAT DIGEST AGAR AT PH 7.6
——	Blank	1300	
5 days	Ordinary CS_2	2900	
60 days	Ordinary CS_2	450	350
30 days	Freshly distilled	620	
45 days	Freshly distilled	360	100
45 days	Freshly distilled	350	270

Once when this procedure was modified by the addition of intermittent heat exposure, complete sterilization was obtained. During the first week of a thirty-day storage period, the sealed tube, containing the powder under freshly distilled CS_2, was kept at a temperature of 56° for a total of thirty-five hours in five intermittent periods. The effect of this latter treatment on proteolytic activity was not ascertained, although it was shown that five- to six-day contacts did not diminish activity.

2. DEFINITION OF STANDARD ACTIVITY

Drug houses have assayed papain preparations by a method involving the digestion of raw meat. It was considered desirable to add another definition of activity based on a more uniformly reproducible substrate and based on a definite chemical change. For this purpose "Coignet's Silver Label Gelatine" was used and the extent of proteolysis ascertained by formol titrations before and after digestion.

Two further methods of assay do not furnish as reproducible definitions of activity since they were more directly intended as methods adapted to the other phases of these studies. The first, the determination of "fibrin digestion time," provides only an approximate estimation of activity, but is relatively simple and convenient. The second, termed the photographic film method, is more exact and gives results in direct units of activity, but must be referred to the activity of some standard powder.

TABLE II

MEDIUM	PHOTOGRAPHIC FILM UNITS PER GRAM OF PAPAIN POWDER
Distilled water	220
Sodium citrate solution (1%)	1,000
HCN solution (0.15%)	1,850
H_2S solution (0.15%)	8,100

In each of these methods, sodium citrate was used since it is one of the more convenient of the socalled "activators." The relative influence of such substances is indicated by figures shown in Table II.

With fibrin digestions the same order of relations is obtained but the differences are not so marked.

Commercial Assay.—The method employed by Parke Davis and Company in standardizing their papain preparations is as follows, according to their description. Ten grams of shredded lean raw beef is mixed with 0.325 gm. of papain and 50 c.c. of water. Digestion is carried out for six hours at 52-55°, the bottle being shaken gently for one minute every fifteen minutes. At the end of digestion the contents are poured into graduated tubes and allowed to settle. With standard, market preparations the undigested residue measures about 7 c.c.

Formol Titrations of Gelatine Digestions.—Five per cent solutions of air-dried gelatine containing 13.5 per cent moisture (determined by drying to constant weight at 110°) were prepared by heating at 37.5° and were always used immediately. Twenty c.c. of this solution was mixed with 18 c.c. of a 5 per cent solution of sodium citrate crystals (U.S.P.) and 2 c.c. of N/10 NaOH and the mixture brought to a temperature of 37.5° in a thermostat, whereupon 20 c.c. of a 2.0 per cent unfiltered suspension or solution of the enzyme preparation was added. After digestion for ninety minutes, 50 c.c. of the mixture was withdrawn and added to 50 c.c. of 37 per cent formaldehyde solution not quite neutralized to phenolphthalein. Using phenolphthalein as an outside indicator, this mixture was titrated with N/10 alkali and the titration of a blank subtracted. Blanks were obtained by carrying out the same incubation with boiled enzyme preparations. The activity of stock preparations of Fairchild's trypsin and of Parke Davis' untreated papain is represented by a titration change of 10.80 c.c. and 3.70 c.c. N/10 alkali, respectively. The activity of two lots of Parke Davis' sterilized papain was represented by a titration change of 2.30 c.c. N/10 alkali. Moisture content of the untreated trypsin and papain was 7.2 per cent and 5.9 per cent, respectively, as determined by drying to constant weight at 110°.

Determination of "Fibrin Digestion Time."—Glycerine stored fibrin of beef blood, in amounts corresponding to 40 or 50 mg. when oven-dried, was washed free of glycerine, shredded with a scalpel and the moist fibrin introduced into 10 c.c. of digestion mixture. Digestions in duplicate or triplicate were carried out in a 1.0 per cent solution of sodium citrate crystals (U.S.P.) at a temperature of 40°. The time for an estimated disintegration of 70 per cent of the shreds is taken as the "fibrin digestion time." After disintegration is once evident, complete disintegration follows rapidly. Disintegration without complete solution is the usual course with papain digestions at this temperature. At higher temperatures, 70°, for instance, there is no residue of disintegrated shreds. The standard preparations showed the following "fibrin digestion times": 1-250 Parke Davis' papain (sterilized), 1.8 hr.; 1-250 Parke Davis' papain (untreated), 2.0 hr.; 1-1,000 Fairchild's trypsin, 0.6 hr.

Photographic Film Method.—The procedures described by Gates[6] and by Gilman and Cowgill[7] have been adapted to the requirements of these studies. The method has the advantage of being relatively quantitative and rapid at very

low activity concentrations and is uniquely suited to the determinations of proteolytic activity at concentrations (1-15,000 papain) which approach the conditions of clinical use.[2] Mechanical features were essentially the same as used by these authors except that cheap alloy wedding rings of 19 mm. inside diameter were used in place of copper wire rings. Eastman Dupli-tized Superspeed X-ray films, sensitized by fifteen seconds exposure to a 15 watt Mazda light bulb at a distance of 26 inches, were developed for two minutes in Eastman x-ray developer and immersed in the fixing solution for four minutes. The gelatine emulsion was stripped from one side of the film with warm water, precautions being taken to leave the other side unaffected. As the films were of 14 by 17 inch dimensions, they furnished a considerable number of single strips of reasonably uniform resistance. Digestions were carried out in a thermostatically regulated water-bath controlled to 0.05°.

Results were arbitrarily expressed on the basis of a definite activity concentration, i.e., that concentration which will convert the photographic film to one-half of its original photometric reading upon digestion for forty minutes at 27.50° in a solution of 1 per cent sodium citrate crystals (U.S.P.). This concentration being taken as unity, the unitage per gram of powder is simply the dilution to which the powder must be carried before its solution reaches this point of activity. The activity of Parke Davis' papain (untreated), which serves as the standard of reference, is set at 1,000 units per gram (air-dried). Accordingly, one gram when diluted to 1,000 c.c. represents a solution of exactly the activity necessary to convert the photometric reading to one-half the original reading. The resistance of various films will diverge considerably from this arbitrary standard and must be calibrated with each series of determinations.

In the study by Gilman and Cowgill with pepsin the photometric change was directly proportional to the logarithm of enzyme concentration. In these studies, the same type of monomolecular reaction was approximated with trypsin but not with papain. With papain, the concentration range between total digestion and zero digestion was very much sharper. This necessitated a greater number of trial assays but, correspondingly, increased the order of accuracy.

The unitage of the standard powders by this method is as follows: Parke Davis' papain (untreated), 1,000; Parke Davis' papain (sterilized), 1,100; Fairchild's trypsin, 24,000.

The Casein Digestion Test.—The U.S.P. or Fuld-Gross method is not suited to papain determinations because of the insoluble products formed by the action on casein.

SUMMARY

Filtered glycerin extracts of papain evidence sufficient instability to preclude their clinical use. Filtered and precipitated powders constitute a product suitable for the purposes described. The activity of such powders is defined according to three procedures. Prolonged treatment with CS_2, as described by Vandevelde, is not adequate for the sterilization of heavily infected papain powders.

This work has been carried out in conjunction with the Department of Surgery and I wish to acknowledge the various types of assistance which they have rendered.

REFERENCES

1. Ochsner, A., and Garside, E.: The Use of Digestants in Preventing the Formation of Adhesions, Surg. Gynec. Obst. *in press.*
2. Walton, R. P.: Behavior of Papain in the Peritoneal Cavity, J. Pharmacol. & Exper. Therap. **43**: 487-497, 1931.
3. Walton, R. P.: Trypsin Preparations Suitable for the Prevention of Adhesions, J. Pharmacol. & Exper. Therap. **40**: 403-411, 1930.
4. Vandevelde, A. J. J.: La stérilisation des farines et des enzymes à l'état pulvérulent. (III.) Acad. roy. de Belg. Bull. de la cl. d. sc. (5) **16**: 585-581, 1930.
5. Vandevelde, A. J. J.: La stérilisation des farines. (II.) Acad. roy. de Belg. Bull. de la cl. d. sc. **5**: 383-392, 1919.
6. Gates, F. L.: A Method of Proteolytic Enzyme Titration, Proc. Soc. Exper. Biol. & Med. **24**: 936-937, 1926-27.
7. Gilman, A., and Cowgill, G. R.: The Determination of Peptic Activity, J. Biol. Chem. **88**: 743-752, 1930.

LABORATORY METHODS

NOTE ON THE CALCULATION OF URINE SOLIDS*

By Allan Winter Rowe, Ph.D., Boston, Mass.

THE amount of solid matter dissolved in the twenty-four-hour urine elimination is a rough measure of the nutritional level. The actual determination, a necessity in certain types of metabolic study, is attended by no little difficulty if error is to be reduced to inconsiderable proportions. Evaporation in vacuo at room temperature is time-consuming, and the last traces of water are difficult to remove. The removal of water by heating, on the other hand, leads to chemical decomposition of the nitrogen-containing substances—primarily urea—with a loss of ammonia and carbon dioxide for which allowance must be made by additional analyses. Neither procedure is adapted to clinical use and for many years the estimation of the specific gravity correlated with the volume has been the usual simple expedient adopted.

The specific gravity of a complex solution, such as is the urine, is a summation of the relative amounts and densities of the various dissolved substances. Further, in dilute solutions the relationship of concentration to specific gravity approaches a linear function. From this fact, if the composition of the solution were fairly constant in the relative proportions of the several constituents, a fairly constant ratio should exist between the specific gravity and the actual amount of dissolved matter. An early recognition of this probability led to investigation, and several decades ago a coefficient was determined which is known usually as Haeser's, Roberts', etc., all resting on the basis of the simple arithmetical relationship—

$$K = \frac{\text{Total Solids per liter}}{1000\ (\text{Specific Gravity}—1.000)}$$

For measurements at 15.5° C., the earlier standard temperature, "K" was found to approximate the value of 2.33. Nearly thirty years ago, Long[1] revised this value by an experimental study and offered the ratio, K = 2.6 when specific gravities are determined at 25° C. A simple computation demonstrates that the two coefficients are in complete numerical agreement, the larger value of Long compensating for the lower specific gravity produced by thermal expansion of the solution. Both Long and his predecessors selected so-called "normal" urines for their investigations, a fact which led to an artefactual constancy in their experimental results. The two chief constituents of the urine in point of amount are urea and sodium chloride. Weight for weight, at concentrations found in urine, the salt influences the specific gravity between two and three

*From the Evans Memorial, Massachusetts Memorial Hospitals, Boston, Mass.
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times as much as does the urea. For example, a 2 per cent solution of the latter has a specific gravity of 1.0058, of the former 1.0146. Since together these two substances constitute from 50 per cent to 80 per cent of the total dissolved matter, they conjointly exercise the principal influence in determining the density; as their several influences are so divergent, due cognizance must be taken of their ratio. In the "normal" urine this assumes a value of approximately two parts of urea to one of sodium chloride.

To reduce these influences to arithmetical terms, the writer has made a series of measurements of densities of a group of solutions containing the two substances in varying proportions.

The desired amounts of urea, sodium chloride, and water were weighed, on balances sensible to 1 centigram, into stoppered Erlenmeyer flasks of 1 liter capacity, the final weight of the solution being 500 grams. After complete solution and uniform concentration had been secured by prolonged shaking, exactly 250 grams of this solution was diluted with an equal weight of water. After thorough mixing, the operation was repeated to produce a more dilute solution and that in turn gave a fourth. Recognizing that an initial error would vitiate an entire series, a duplicate of some one of the dilute solutions in each series was supplementarily made by careful weighing and its specific gravity was compared with that of the original solution made by dilution. These checks in every instance justified the confidence placed in the original observations. The urea was of "highest purity" quality, the sodium chloride of equally high grade and yielded on analysis less than 0.1 per cent of moisture. The solutions were made in distilled water.

As soon as prepared the solutions were placed in dry, clean, glass-stoppered bottles and these stored in a cool room. The densities were determined inside of a few hours, using a carefully standardized Westphal balance. These balances are commonly adjusted to give their 0 reading at 15.5° and as the majority of urinometers on the market are constructed for this temperature, it was taken as the standard. As the weather was very warm, some little difficulty was experienced at first in holding the solutions at the proper temperature. The substitution of a silvered Dewar cylinder of some 200 milliliters' capacity for the usual glass receptacle, made it easily possible to maintain constant thermal conditions. A carefully standardized thermometer gave the temperature of the solution; the use of small glass stirrers ensured thermal homogeneity. The initial results are collected in tabular form (Table I).

A number of the higher concentrations fall outside the range of physiologic probability and are included solely to define the trends of the curves. The values in brackets at the low concentrations are extrapolated, a warrantable procedure as the relationships are known to be substantially rectilinear at these levels.

A review of the data shows some interesting features. The "K" values approximate constancy for any given ratio; within the usual concentration limits, the variation is of the order of the ordinary observation error. The coefficient of the solution with the ratio of the "normal" urine, i.e., 2 to 1, is the figure of the earlier experimentally determined constant. Seemingly, at these concentrations the algebraic summation of the other urinary constituents produces a solution of a density approximating that of the urea salt mixture. That this would hold

TABLE I

SPECIFIC GRAVITIES AT 15.5°C. AND "K" VALUES FOR SOLUTIONS OF UREA AND SODIUM CHLORIDE

UREA %	UREA	UREA / NaCl = 12/1	8/1	6/1	4/1	3/1	2/1	1/1	0.5/1	0.25/1	NaCl EQUIVALENT %
16	1.0462 3.62	—	—	—	1.0742 2.90	—	—	—	—	—	1.1190 1.50
8	1.0232 3.53	1.0267 3.33	1.0294 3.15	1.0315 3.05	1.0366 2.83	1.0428 2.60	1.0518 2.43	1.0808 2.14	1.1420 1.93	— —	1.0585 1.45
4	1.0115 3.52	1.0132 3.33	1.0146 3.13	1.0157 3.02	1.0184 2.77	1.0211 2.58	1.0257 2.39	1.0402 2.07	1.0699 1.84	1.1321 1.71	1.0291 1.41
2	1.0058 3.47	1.0067 3.26	1.0074 3.08	1.0078 3.01	1.0093 2.71	1.0105 2.57	1.0130 2.34	1.0199 2.05	1.0351 1.77	1.0650 1.64	1.0146 1.39
1	1.0029 3.45	1.0034 3.20	1.0037 3.05	1.0039 3.00	1.0046 2.73	1.0053 2.53	1.0065 2.32	1.0099 2.05	1.0175 1.74	1.0325 1.59	1.0073 1.38
0.5	(1.0014[5]) 3.45	(1.0017) 3.19	(1.0018[5]) 3.05	(1.0019[5]) 3.00	1.0024 2.61	1.0027 2.48	1.0033 2.30	1.0049 2.05	1.0087 1.74	1.0162 1.57	1.0037 1.36
0.25	(1.0007[5]) 3.45	(1.0008[5]) 3.19	(1.0009[5]) 3.02	(1.0009[5]) 2.99	1.0012 2.60	(1.0013[5]) 2.47	(1.0016[5]) 2.28	(1.0024[5]) 2.05	1.0043 1.75	1.0081 1.56	(1.0018[5]) 1.35

in urines of markedly abnormal composition is doubtful, but such urines are exceptional.

On the other hand, as was to be anticipated, the "K" value varies over a very wide range as the ratio of the two substances changes. A pure 2 per cent urea solution has a "K" value of 3.47, while the "K" of the equivalent salt solution is but 1.39.

The amount of urea eliminated is directly influenced by the level of protein catabolism, as was shown most graphically by Folin[2] many years ago and since confirmed by a host of investigators. A variety of toxic states increase the urea output, while lowered renal permeability will depress its elimination. The possibility of urea storage is a factor that cannot be discussed here. Sodium chloride in largest measure reflects the level of salt intake. In fasting[3] it sinks to very low levels as well as in a variety of pathologic conditions of which the exudative phase of pneumonia is a striking example.

As the variation with the concentration of the "K" value for any given ratio has already been shown to be of but minor proportions, it is a simple matter to plot the curve of average "K" values over a wide range of ratios. For the sake of convenience these may be reduced to tabular form, using round numbers only in recognition of the very approximate character of the data.

TABLE II

"K" VALUES FOR VARIOUS RATIOS OF UREA AND SODIUM CHLORIDE

Urea / NaCl	=	100	50	25	12	10	8
K	=	3.5	3.4	3.3[5]	3.2[5]	3.2	3.1
		6	4	3	2	1	0.5	0.25
		3.0	2.7	2.5	2.3	2.0[5]	1.8	1.6

To calculate the total solids per liter, the following formula is derived from that first given.

Total Solids = 1000 K (Specific Gravity—1.000) per liter. Substitution of the actual volume in c.c. for the 1000 gives a first approximation of the solid elimination in the specimen. To apply, determine the urea and sodium chloride by any of the standard quantitative procedures and use the "K" value indicated by their ratio.

Two points of exception should be noted in applying the formula. The presence of glucose in appreciable amounts necessitates a correction. This can most easily be applied by deducting from the observed specific gravity an amount

TABLE III

APPROXIMATE CORRECTION FOR SPECIFIC GRAVITY $\frac{15.5°}{15.5°}$ OF GLUCOSE SOLUTIONS

% Glucose	1	2	3	4	5	7.5	10
Correction	–.004	–.008	–.012	–.016	–.020	–.030	–.040

equivalent to that produced by the amount of sugar present. Long[1] suggested this expedient for the sodium chloride, using a modified coefficient for the remaining solids.

A second source of error is the socalled "ketone" bodies with acetone as the most disturbing factor. The relative amounts of the several substances and the degree of salt formation of the two acids would be very variable and preclude the application of a simple correction such as is appropriate in the case of sugar.

With these two exceptions, the use of the coefficients from Table II permits of a rough estimate of the dissolved matter of the urine on a parity in accuracy with the measurement of the specific gravity.

Even so rough an estimate has a certain value for clinical interpretation and patently offers a definite advantage over the time-honored practice.

REFERENCES

1. Long, J. H.: On the Relation of the Specific Gravity of Urine to the Solids Present, J. Am. Chem. Soc. **25(3)**: 257-262, March, 1903.
2. Folin, O.: Laws Governing the Chemical Composition of Urine, Am. J. Physiol. **13(1)**: 66-115, February, 1905.
3. Benedict, F. G.: The Influence of Inanition of Metabolism, Carnegie Inst. Wash. Pub. No. 77, 542 pp., 1907.

A NONGLUCOSE REDUCTION PRESENT IN NORMAL AND INCREASED IN NEPHRITIC BLOOD*

By Rawson J. Pickard, M.D., Leo F. Pierce, Ph.D., C. S. Marsden, Jr., R. K. Tanaka, and H. A. Townsend, A.B., San Diego, Calif.

INCREASE of the blood sugar in cases of nephritis, first reported by Myers[1] in 1916, has been repeatedly confirmed, but by the use of improved methods, we now recognize a lower normal for blood sugar, and recognize too that the methods in general use clinically give glucose figures that are still too high, as they include another reduction than that of glucose. However since the nonglucose reduction is small and practically constant in amount, clinical interpretation is not affected.

The true glucose content of the blood may be distinguished from the apparent glucose measured by oxidation methods by the loss in reduction after fermentation by yeast. Somogyi,[2] Fontès and Thivolle,[3] and others have shown that yeast completely removes glucose from the blood within a few seconds and at ordinary temperatures, and that there is no other action of the yeast on the blood affecting reduction, as the same results may be obtained by allowing the yeast to act on the neutralized deproteinized filtrate. Somogyi and Kramer[4] found the true sugar the same with various methods of estimation, while the nonglucose reducing body varied with different oxidizing agents.

The nonsugar reducing substance, estimated as glucose, was found by Somogyi and Kramer to be quite uniform (23 to 31 mg.), averaging 27 mg., using tungstate precipitation and the Shaffer Hartmann method. With the Folin-Wu method, West, Scharles, and Peterson[5] found the nonglucose reducing body 20 mg., and Benedict with the Benedict copper reagent found 11.7 mg. average. Bigwood and Wuillot[6] found 14 to 25 mg. nonglucose reducing substance, using the Hagedorn and Jensen method (Table I). Controlled by fermentation, it has been shown that the nonglucose reducing body is precipitated by mercury,[8] the filtrate containing only glucose as reducing substance. Thus the protein precipitant as well as the oxidizing agent are essential factors in the amount of reduction found in the blood and ascribed to the included glucose.

TABLE I

REDUCTION IN BLOOD FILTRATES AFTER REMOVAL OF GLUCOSE BY YEAST "GLUCIDE X"

Somogyi and Kramer[4]	av. 27 mg. Shaffer Hartmann
West, Scharles, Peterson[5]	av. 20 mg. Folin-Wu
Benedict[7]	av. 12 mg. Benedict copper method
Benedict[7]	av. 22 mg. Flin-Wu
Bigwood and Wuillot[6]	av. 19 mg. Hagedorn Jenson
Folin and Svedberg (diabetics)	av. 20 mg. Folin-Wu
Folin and Svedberg (diabetics)	av. 9 mg. Folin
Present report	av. 21 mg. Folin-Wu

*From the Scripps Zoological Hospital and Research Institute, San Diego, California, and The Grace Deere Velie Metabolic Clinic, Carmel-by-the-Sea, California.
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Fontès and Thivolle give as rules for the control of a method for the estimation of blood glucose: (1) to remove the glucose only (by yeast), (2) precipitate the proteins, (3) find no glucose in the filtrate, (4) on adding glucose to the filtrate to find it quantitatively. Their method they state fulfills these rules. Bigwood and Wuillot conclude "there is in the plasma and in whole blood reducing substances, probably glucidic, which yeast does not attack. These substances do not include uric acid or creatinine" except with very high nitrogen retention. Fontès and Thivolle think normal blood contains besides free glucose a non-glucose reducing substance which may be a "glucidic ether," provisionally called "glucide X," which is precipitable by mercury. The ether element is hypothetical, but the glucid nature they assume because of: (1) its destruction by yeast, (2) the increase of glucide X along with free glucose by the action of adrenalin, (3) the decrease of glucide X with the use of insulin.

We have then, normally, about 20 mg. of reducing substance, glucide X, which is oxidized and estimated as, and with glucose by the Folin-Wu method in the tungstate filtrate, the various methods of estimation corresponding in this finding within the limits of error. Glucide X has been found a nonfermentable remainder in the tungstate filtrate after the destruction of the free glucose by yeast. The reduction, expressed as glucose, shows a variation in sensitivity to glucide X by the reagents of the different methods. Zinc and mercuric precipitants precipitate, or change, glucide X, leaving only the free glucose as reducing body and checking against the yeast method. The copper reagent of Fontès and Thivolle is so modified that it is not sensitive to glucide X after the action of yeast, although this body can be shown in the tungstate filtrate they use, after yeast fermentation, by other methods of oxidation. They measure glucide X by the difference in reduction between the tungstate and zinc filtrates. Glucide X is changed then by yeast, but not destroyed by it, and Fontès and Thivolle's method which is insensitive to this body after yeast, thus shows this interesting change in this reducing body, although they consider it fermented and destroyed by yeast, contrary to the finding of most writers. Our results were nearly all in accord with the findings of others, that glucide X reduces the Folin-Wu copper solution after the removal of glucose by yeast, and we estimated it thus. However we had some irregular results which are perhaps explained by the occasional modification of glucide X by yeast, preventing its action on the Folin-Wu reagents, a modification which regularly prevents its reduction of the copper solution of Fontès and Thivolle. Table II shows that this body cannot be demonstrated in some bloods after fermentation.

The difference between the Benedict modification of the Lewis-Benedict method[8] and the Folin-Wu, and the reducing body or bodies in column 3, Table II, is the subject of the present report. In the course of the laboratory examination of a case of nephritis, we found a considerable discrepancy between the blood sugar estimations by these two methods. The patient was able to be at his office most of the time, although constantly showing large amounts of albumin with a sediment of granular and cell casts in his urine; his blood urea N varied from 25 to 36 mg. per 100 c.c. At the higher figure he had vertigo and headaches and was compelled to rest. His blood sugar by the Folin-Wu method had been about 100 mg. The last time we saw him his blood urea N was 31, sugar (F-W) 98 mg.,

TABLE II*

GLUCIDE X SOMETIMES FERMENTABLE

CASE	F-W†	L-B	Y-REDUCTION	AFTER FERMENTATION BY YEAST		
				TIME	F-W	L-B
Hypertrophied prostate	244	241	0	30 min.	0	0
Glomerulonephritis	98	173	75	30 min.	0	79
Chronic nephritis	100	137	37	30 min.	0	40
Early interstitial nephritis	97	120	23	1 min.	30	—
				hour	28	—
Same, week later	100	144	44	1 min.	22	80
				20 min.	0	80
				hour	0	65
				day	0	70
Nephritis, hypertrophied prostate	144	200	64	1 min.	30	
				hour	0	
Interstitial nephritis	125	190	65	1 min.	29	
				hour	30	
				3 hr.	29	
				18 hr.	18	
Interstitial nephritis	95	230	135	1 min.	0	
Interstitial nephritis	150	240	90	1 min.	0	

*Standard methods in standard quantities were used throughout this work (no micro-methods). The Benedict modification as in Underhill (ref. 8). Baker chemicals. At least three-fourths of the figures in the tables are the average of duplicate tests run by different individuals.

†F-W = Folin-Wu blood sugar method, L-B = Benedict modification of the Lewis-Benedict method. All figures milligrams per 100 c.c. of blood.

and 173 by the Benedict modification, showing a reducing body of 75 mg. estimated as glucose, a difference far beyond any error inherent in the methods. The reducing body was not glucose, it did not affect the Folin-Wu copper reagent, and when the blood glucose was removed by yeast (thirty minutes), the Folin-Wu gave a zero reading while the picrate method gave a reading practically equal to the previous difference. (This later proved to be an exceptional result.)

It has been shown[9] that the Benedict modification averages about 10 per cent higher than the Folin-Wu with dextrose solutions. With blood, the higher readings of the Benedict modification have caused it to be dropped and perhaps have somewhat discredited the simple and reliable picrate method of Myers which we have found checks so accurately with the Folin-Wu, that we have used it with the latter as a check on the Benedict modification for the estimation of the y-reduction, which thus evidently is not due to the picrate alone. It was suggested by Professor Myers (personal communication) that the higher reading might be due to an increased sensitivity to "creatinine" of the Benedict modification over the original Benedict picrate method as modified by Myers and Bailey. The latter gives a creatinine reduction equal to that of an equal amount of glucose, Myers has proved, and therefore negligible in any but exceptional bloods. On his sug-

gestion we therefore ran controls with the Benedict modification on glucose solutions to which creatinine was added, 1, 5, and 10 mg. creatinine, with 100 mg. glucose gave 105, 108, and 107 average readings.

Table III gives the "y-reduction" in 25 normal bloods, taken after an eighteen-hour carbohydrate fast from students of athletic type at San Diego

TABLE III
Y-REDUCTION IN NORMAL BLOODS

STUDENTS	L-B	F-W	Y-REDUCTION	STUDENTS	L-B	F-W	Y-REDUCTION
Br	149	82	67	Mg	114	86	28
Su	146	102	44	Wi	139	71	68
Wa	136	82	54	Wo	151	83	68
Ra	134	87	47	Ja	138	85	53
Ro	131	86	45	To	129	69	60
To	107	82	15	Jo	113	79	34
Je	114	86	28	Ph	130	78	52
Ma	114	83	31	OF	118	77	41
Mg	106	83	23	Co	124	79	45
Md	103	76	27	Ar	120	80	40
Ro	89	75	14				
Bo	129	92	37				
Hu	97	80	17				
Ke	119	71	48				
Ma	104	80	24				

State College. The y-reduction varies considerably in normal blood, from 14 to 68 mg., average 40.4 mg. Note that by the Benedict modification several of the normal students would have been considered as having glycemia. Table IV gives the y-reduction in the blood of patients with nephritis as evidenced by finding albumin and casts. Table V lists the negative findings. Among the nephritics of Table IV, 9 of the 41 examinations gave glucose over 120 mg. by the Folin-Wu method; confirming previous reports of high blood sugar in nephritis, the picrate method shows 31 over 130 mg. Glycosuria was occasional, in only one of these cases.

The y-reduction in 7 tests on 4 patients with severe nephritis, heading Table IV, averages 108.7 mg., the average y-reduction of the other bloods in Table IV is 48.7 mg., and the average of all samples is 58 mg., much over the average of the normal bloods (40.4 mg.). That this increase is not due to the creatinine bodies is shown by comparison with the creatinine findings, Table VI. The y-reduction is not necessarily due to one substance, and that this is possibly true of nephritic blood is suggested by the abnormal ratio of urea nitrogen to the nonprotein nitrogen in 7 estimations, 6 cases, heading Table IV; the average urea nitrogen is 35 mg., nonprotein nitrogen 110.9 mg.; ratio 1 : 3.2. It was our intention to learn the clinical significance of the higher reduction in nephritis by interpolating this finding in the "staircase" table of V. C. Myers, adding to the creatinine, uric acid and urea which formed his table, lowered phthalein output and fixation of specific gravity. We soon found this program too ambitious in a city without the facilities of a teaching hospital.

TABLE IV*

CASES AND DIAGNOSTIC DATA	DAYS	BLOOD CHEMISTRY		REDUCING SUBSTANCES F-W	L-B	Y-REDUCTION
Chronic nephritis, no albuminuria, few granular casts, fixed Sp. Gr. Occasional glycosuria. (Dr. St. Sure: "R")		urea N NPN	23 77	109	135	26
Chronic nephritis, albumin 4+, hyaline and granular casts 4+. (Dr. Stigall: "C")		urea N NPN creat.	12 95 1.3	100	293	193
Same, diet with reduced protein, 2 weeks		urea N NPN	16 105	105	150	45
Same, third week.		urea N NPN	24 53	136	200	64
Uremia, coma, albumin 4+, casts 4+, moribund. (Dr. Barclay: "W")		urea N NPN creat.	140 340 6	107	224	117
General arteriosclerosis, chronic nephritis, ascites. P.S.P. 11 per cent. (Dr. Potter: "Br")	Feb.	urea N creat.	64-80 3-3.5			
	Apr.	urea N	60	192	260	68
	May			179	227	48
Trace albumin, no casts. Died in December.	Oct.	urea N creat. ur. ac.	37 1.4 3.2			
Arteriosclerosis, hypertension, cerebral hemorrhage, death. (Dr. Newmann: "M")		urea N NPN creat.	20 75 1.8	154	380	226
Hypertrophic prostate, retention, albumin and granular casts. P.S.P. 60 per cent. (Dr. Hall: "Sw")	1	urea N	25	125		
	30			95	135	40
	48	urea N creat.	23 1.6	122	202	80
	55	urea N creat.	25 2.5	105	123	18
	60	urea N	15	85	149	64
	67	urea N	15	97	190	93
Hypertension, myocarditis, albumin and casts. (Dr. Hall, Naval Hospital: "Co")	1	urea N	32	96	122	27
	3			85	124	39
	20			100	123	23
Hypertrophy of prostate, obstruction, cystitis, pyelitis. (Dr. Hall: "Fw")	1	urea N creat.	32 2.5	75		
	7	urea N creat.	28 1.7	72	134	62
	14	urea N creat.	32 2.5	91	148	57
Chronic nephritis, myocarditis, recovering from hemiplegia, albumin, granular casts. (Dr. Hall: "Kr")	1	urea N creat.	28 1.5	81	127	46
	3			164	300	136

TABLE IV (Continued)

CASES AND DIAGNOSTIC DATA	DAYS	BLOOD CHEMISTRY		REDUCING SUBSTANCES		
				F-W	L-B	Y-REDUCTION
	13			118	181	63
	22	urea N creat.	25 2	87	139	52
	40	urea N creat.	12 1.7	76	145	69
Arteriosclerosis, hypertension, hypostatic pneumonia, death. Age 68. (Dr. Stevenson: 31170)		urea N	7	94	156	62
Persistent secondary anemia, low fixed Sp. Gr. (Dr. Kennell: "CR")				100	144	44
Arteriosclerosis, syphilis, chronic nephritis myocarditis, albumin, hyaline and granular casts. Age 63. (Dr. Stevenson: 28833)				110	127	17
Bronchopneumonia, lung abscess, age 78, albumin, granular casts. (Dr. Stevenson: 28676)		urea N creat. PSP	11 1.7 46%	104	133	29
Hypertrophy of prostate, trace albumin, age 55. (Dr. Stevenson: 28757)		urea N creat. PSP	15 1.7 37%	111	139	28
Salpingitis, albumin, granular casts, age 32. (Dr. Stevenson: 28487)				93	187	94
Syphilis, meningitis, albumin hyaline, granular casts, age 64. (Dr. Stevenson: 28841)		urea N creat.	30 2.	82	119	37
Cancer breast, trace albumin, casts, age 67. (Dr. Stevenson: 28251)				110	139	20
Cellulitis hand, syphilis, trace albumin, casts, age 59. (Dr. Stevenson: 27901)				86	178	92
Endometritis, albumin, many hyaline, few granular casts, age 29. (Dr. Stevenson: 28500)				114	134	20
Luetic aortitis, trace albumin, casts, age 67. (Dr. Stevenson: 28552)		urea N NPN creat.	10 31 1.7	104	123	19
Sinus operation, hemorrhages, convulsions. (Mercy Hosp.: 438)		NPN creat.	136 3.2	130	167	37
Mastoiditis, age 42, trace albumin. (22687)				71	114	43
Acute cholecystitis, age 54, albumin, granular casts (28944)				97	130	33
Arthritis, age 35, trace albumin hyaline casts (28829)		urea N creat.	7 1.6	93	134	41
Chronic nephritis, granular casts, albumin. (FK)				98	146	48
Cancer prostate, anemia, 20 per cent Hb. (Dr. Russell: "O'D")		NPN creat.	82 1.8	100	137	37
Infectious jaundice, albumin granular casts. (Dr. Stealy: "D")		NPN urea N creat.	80 53 1.2	100	137	37

*In parentheses, name of referring physician and identification of patient. All figures for blood chemistry in milligrams per 100 c.c. blood. F-W, glucose by the Folin-Wu method. L-B, same by Benedict modification of Lewis-Benedict method. Alb., albumin; gr., granular; h., hyaline; c., casts.

TABLE V

CASES WITH NEGATIVE FINDINGS	DAYS	BLOOD CHEMISTRY	REDUCING SUBSTANCES		
			F-W	L-B	Y-REDUCTION
Toxic adenoma thyroid, albumin, granular casts, age 47. (28912)			100	99	–
Essential hypertension, trace albumin, casts, age 40. (28889)		urea N 22-25	128	135	7
Chronic nephritis, arteriosclerosis, myocarditis, albumin, few hyaline casts, age 67. Died. (28411)			133	136	–
Arteriosclerosis, cardiac decompensation, albumin, hyaline casts, age 59. (28705)			156	156	–
Prostatic hypertrophy, age 89, trace albumin. (28767)		urea N 15	129	121	– 8
Cholecystitis, trace albumin, casts, age 57. (31835)			166	150	–16
Pleurisy, fibroid tbc., albumin, casts, age 70. (28493)			132	119	–13

TABLE VI

CREATININE	Y-REDUCTION	CREATININE	Y-REDUCTION	CREATININE	Y-REDUCTION
1.	0	1.7	69	2.5	57
1.2	37	1.7	29	3.2	68
1.3	193	1.7	28	3.2	48
1.5	46	1.8	37	3.2	47
1.6	80	1.8	226	6	112
1.6	41	2	52	6	117
1.7	19	2	37		
1.7	62	2.5	18		

Table VII of 27 blood samples from 9 patients with nephritis shows the relation of the y-reduction to glucide X. Column 1 gives the blood glucose estimated by the Folin-Wu method, Column 2 the reduction by Folin-Wu after two-hour fermentation by yeast expressed as glucose, "glucide X"; the difference is the true (free) glucose. The average of glucide X in these nephritic bloods, 20.8 mg., shows no increase over normal blood, nor is the true glucose high except in two samples (different cases). There follow the reduction by the Benedict modification, its increase over the Folin-Wu, or the "y-reduction": the attempt to estimate the reducing bodies by the picrate method in blood from which the true glucose was first removed by yeast gave very irregular results (Column 7), perhaps an adsorptive effect on the picrate coagulum from yeast. Nevertheless the average was the same as the average of the sum of the two non-glucose reductions.

Somogyi[4] found glucide X uniform in quantity independently of the blood sugar level in health and disease, except in cases of severe diabetes when glucide X diminished as the high blood sugar persisted. Nor do we find glucide X

TABLE VII

F-W GLUCOSE	F-W AFTER YEAST FERMENTATION "GLUCIDE X"	TRUE GLUCOSE	L-B GLUCOSE	Y-REDUCTION	TOTAL, BOTH NON-GLUCOSE REDUCTIONS	L-B AFTER YEAST
105	18	87	123	18	36	—
100	14	86	123	23	37	70
99	15	84	125	26	41	67
104	21	85	127	23	44	36
92	20	72	123	31	51	48
96	27	69	123	27	54	82
84	23	61	115	31	54	100
86	17	69	125	39	56	34
84	22	62	124	40	62	67
100	22	78	144	44	66	80
81	18	63	128	65	—	—
106	20	86	—	—	—	—
72	18	54	134	52	70	81
101	20	77	149	48	72	81
91	18	73	148	57	75	63
95	37	58	136	41	78	120
84	18	66	149	65	82	74
73	21	52	141	68	89	81
76	21	55	145	69	90	84
113	22	85	181	68	90	92
91	—	—	137	46	—	66
97	20	77	168	71	91	106
123	18	105	202	79	97	68
98	13	85	190	92	105	82
73	24	49	169	96	120	80
96	20	76	202	106	126	68
164	31	133	300	136	167	117
Average	20.8			55.5	76.7	77

higher in nephritis, although Linder, Hiller, and van Slyke,[10] using the Folin-Wu method, found the nonglucose reducing body higher in cases of glomerulonephritis, about 40 mg., along with a lowered sugar tolerance and lower sugar threshold. Our findings in the blood of patients with nephritis are occasional high free glucose, normal glucide X, and a considerable increase over normal in such blood of a nonglucose reduction which we call the y-reduction, and which is present in much larger amount than glucide X (estimating as glucose) and may even be in greater reduction value than the glucose itself.

Fontès and Thivolle found glucide X consumed first under the influence of insulin, and more completely than glucose. Tests on a case of diabetes with chronic nephritis under treatment at the Naval Hospital, through the kindness of Dr. Hall, are recorded in Table VIII. The patient had a mild attack of coma during the time of observation. The remainder of fermentation, glucide X, did not disappear in this case when the blood sugar was high, but the y-reduction disappeared and at the highest glucose level, with large doses of insulin, the difference between the two blood sugar methods became a negative one, and this at a glucose level where the picrate method reads 3 per cent higher than the Folin-Wu and 24.5 per cent higher than the actual glucose content.

TABLE VIII

DIABETIC (MA.) COURTESY DR. HALL, U. S. N. HOSPITAL

DATE	F-W	L-B	Y-REDUCTION	GLUCIDE X (F-W AFTER YEAST)
Oct. 3	265	395	130	16
Oct. 6	179	363	174	22
Oct. 13	240	270	30	—
Oct. 20	404	689	285	12
Oct. 27	406	402	—	56
Nov. 3	470	440	-30	20
Nov. 17	420	555	135	22
Nov. 24	393	516	125	
Dec. 1	394	548	154	
Dec. 8	437	660	223	
Dec. 15	352	470	118	

The fact that the y-reduction was demonstrated by a comparison of both recipitant and oxidizing agents leads to inaccuracies which we tried to overcome by finding a more direct method. We found the Myers picrate method gave the same results in normal or nephritic bloods as the Folin-Wu, and used it frequently as a check, so that the y-reduction may be measured by a comparison of picrate methods, the Benedict modification being sensitive to the y-reduction substance together with glucide X and the true glucose, while the other two methods show reduction from only the latter two bodies. We later found the Ionesco[11] method sensitive to all the reductions, like the Benedict modification, and by this method, the y-reduction can be demonstrated present in the tungstate filtrate. In a paper by one of us (R. J. P., read before the California State Medical Meeting, 1931), it was shown that this reduction is present as well in equal amounts in the trichloracetic and m-phosphoric filtrates.

COMMENT

1. True glucose is often increased in nephritis.

2. The nonfermentable remainder estimated by the Folin-Wu method, glucide X, is constant in health and disease. Contrary to most authors, we found glucide X occasionally fermented by yeast. The work of Hubbard and Deegan[12] suggests that it is a higher sugar.

3. The blood glucose may be estimated by either the Folin-Wu, the Myers, or Benedict copper methods without clinical error. When the glucose alone is to be estimated frequently we recommend the Folin-Wu technic as modified[13] for use with 0.1 c.c. of blood. The Benedict modification of the Lewis-Benedict method should not be cited[14] as a method for blood sugar as that will lead into greatest error. It is especially unfair to Dr. Benedict who has substituted an accurate copper method[15] for blood sugar.

4. We describe a reduction in blood which we call the y-reduction, which varies in wide limits in health and disease, but averages much higher in the blood of nephritics and is highest in severe cases of nephritis. This reduction does not seem to have any relation to "creatinine." It may be due to several bodies and the reduction in nephritis may in part be due to a nitrogenous substance.

5. The question of glutathione occurs in connection with these results. Benedict and Newton[7] state that glutathione is one of the chief nonglucose reducing substances using their methods. They further state that it is present in blood to an extent of 50 to 100 mg. per 100 c.c. and that with the Folin-Wu reagents it reacts in such a way as to give a reading in terms to glucose, corresponding to 0.2 the actual glutathione present, or in other words, an excess reduction of 10 to 20 mg. per 100 c.c. All these reactions are fundamentally of the oxidation-reduction type, and there is undoubtedly a fair degree of comparability between results gained by all methods.

Benedict[16] states that in view of later work "a considerable portion of the saccharoid fraction of blood is not represented by glutathione." He likewise says "We are of the opinion that on the average glutathione accounts for little of the saccharoid content of blood." He shows that an average saccharoid content of 19.9 mg. for twenty samples would require a glutathione content of 100 mg. per 100 c.c. of blood, and adds that "There is not satisfactory evidence that blood contains upon an average one-half as much glutathione as this." In a footnote in this same article, he discusses a communication from Downes which gives an average glutathione content of 23.1 mg. in six bloods by the Mason method. Benedict concludes the footnote with "Until more extensive figures are available for human blood by the Mason method and until the accuracy of the method has been further tested, we cannot draw definite conclusions concerning the glutathione content of blood."

Work is now in progress to determine the relation of glutathione to this y-reduction.

REFERENCES

1. Myers, Victor C.: Practical Chemical Analysis of Blood, ed. 2, St. Louis, 1924, The C. V. Mosby Co., p. 86.
2. Somogyi, M.: The Distribution of Sugar in Normal Blood, J. Biol. Chem. **78**: 117, 1928. Reducing Non-Sugars and True Sugar in Human Blood, J. Biol. Chem. **75**: 33, 1927.
3. Fontès, G., and Thivolle, L.: Sur la validité des chiffres de la glucidemie immédiatement réductrice, Bull. Soc. Chim. Biol. VI, VII, VIII, IX. **12**: 264, 270, 278, 283, 1930. I, II, III, Bull. Soc. Chim. Biol. **11**: 146, 152, 159, 1929.
4. Somogyi, M., and Kramer, H. V.: The Nature of Blood Sugar, J. Biol. Chem. **80**: 733, 1928.
5. West, E. S., Scharles, F. H., and Peterson, V. L.: The Determination of True Sugar in Blood, J. Biol. Chem. **82**: 137, 1929.
6. Bigwood, E. J., and Wuillot, Mlle. A.: De l'analyse de la glucidémie par la méthode de Hagedorn et Jensen, Bull. Soc. Chim. Biol. **11**: 1204, 1929.
7. Benedict, S. R., and Newton, E. B.: Studies on Non-Sugar Reducing Substances in Blood and Urine, J. Biol. Chem. **83**: 361, 1929. The Determination of Blood Sugar, J. Biol. Chem. **76**: 457, 1928.
8. Underhill, F. P.: A Manual of Selected Biochemical Methods, New York, Wiley and Sons, 1921.
9. Pickard, R. J., and Pierce, L. F.: Blood Dextrose Determination: A Statistical Comparison of the Folin-Wu Method and the Benedict Modification of the Lewis-Benedict Method, J. A. M. A. **94**: 480, 1930.
10. Hiller, A., Linder, G. C., and van Slyke, D. D.: The Reducing Substances of the Blood, J. Biol. Chem. **84**: 625, 1929.
    Linder, G. C., Hiller, A., and van Slyke, D. D.: Carbohydrate Metabolism in Nephritis, J. Clin. Investig. **1**: 247, 1925.
11. Ionesco-Matiu, Prof., and Vitner, Mlle. M.: Étude comparative de quelques procédés de dosage des glucides sanguins, Bull Soc. Chim. Biol. **12**: 626, 1930.
12. Hubbard, R., and Deegan, J. K.: Experiments with the Non-Glucose Sugar of the Blood and Plasma, J. Biol. Chem. **78**: lvii, 1928.

13. Pickard, R. J., and Pierce, L. F.: Accurate Blood Sugar Determination with 0.1 and 0.05 c.c. of Blood, J. A. M. A. **94**: 1134, 1930.
14. Hawk, P. B., and Bergeim, O.: Practical Physiological Chemistry, ed. 9, Philadelphia, 1927, Blakiston.
15. Benedict, S. R.: The Estimation of Sugar in Blood and Normal Urine, J. Biol. Chem. **68**: 759-767, 1926.
16. Benedict, S. R.: The Analysis of Whole Blood I & II, J. Biol. Chem. **92**: 135, 141, 1931.

A MANOMETER FOR MAGNIFICATION OF BLOOD PRESSURE TRACINGS*

By Robert K. Webster, A.B., and William E. Fry, Indianapolis, Ind.

INTRODUCTION

VARIOUS kinds of instruments for recording pharmacologic tracings make use of levers to magnify these changes when recorded on the kymograph. Whenever changes are relatively slight, as in kidney volume, spleen volume, or nasal mucous membrane volume, it is absolutely essential that these changes be

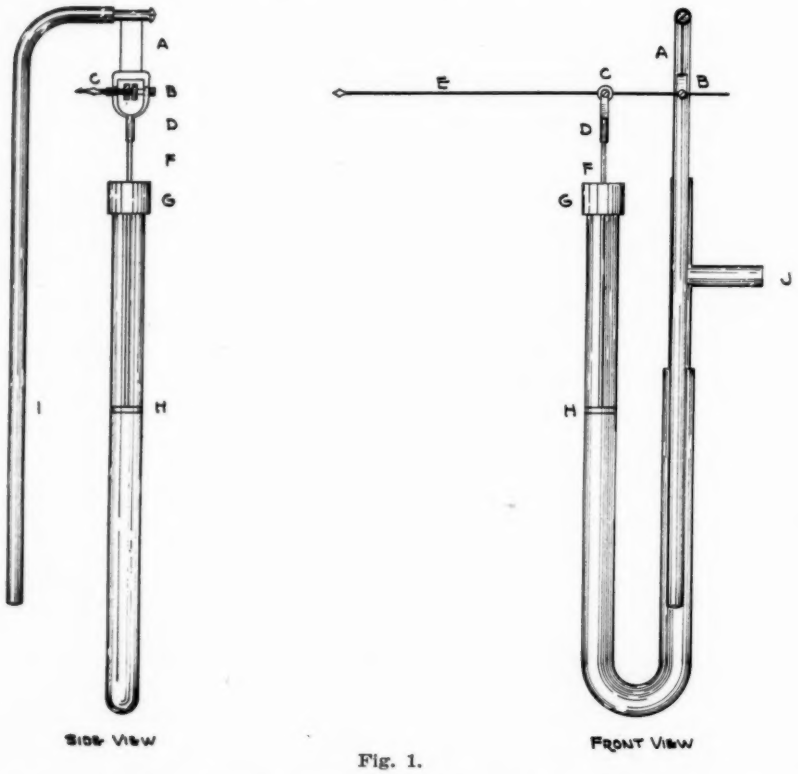

Fig. 1.

greatly magnified by the recording instrument. If this were not accomplished, much greater difficulty in interpreting results would be experienced. It is believed that some such apparatus would be useful for comparative work on blood pressure, or for use when changes in blood pressure are very slight. As a result there has been developed a manometer which has been in use for several months and has been found very satisfactory.

*From Lilly Research Laboratories, Eli Lilly and Company.
Received for publication, September 3, 1931.

DESCRIPTION OF MANOMETER

The usual mercury U-tube manometer was used and the levers were arranged as follows (Fig. 1): *A* is a strip of copper ½ inch by 1 inch, to the lower end of which was welded a needle-point bearing *B* such as is used on heart levers. This strip *A* was fastened at its upper end to a curved rod *I* for attachment to a ring-stand or other support. The lever *E* is of aluminum and passes through another needle-point bearing *C*. This bearing is mounted in a Y-shaped holder *D* made of brass which was as lightly constructed as possible. A hole was drilled in the lower arm of the holder *D* in which was inserted another light aluminum wire *F*. The lower end of the wire *F* is fastened in an ordinary fiber float *H*. Connection to the artery is made in the usual manner from the arm *J*. The cap *G* of the manometer has an extra large opening for the passage of the wire *F* so that there will be no friction between wire and cap. There are three freely movable joints

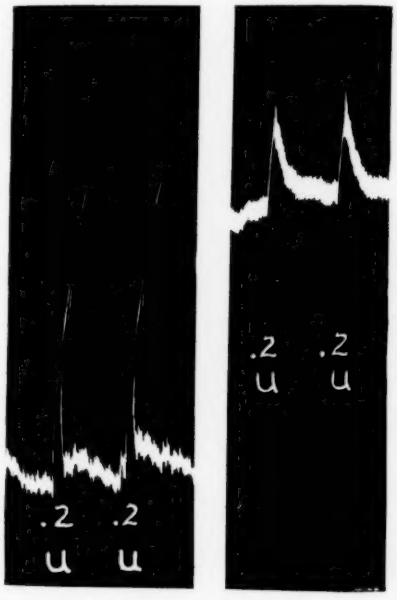

Fig. 2.—Cat, anesthetized with sodium amytal, 65 mg. per kg. Blood pressure recorded from carotid artery. Doses, 0.2-unit of pituitary extract given intravenously.

in the apparatus at *A*, *B*, *C*; however, there is very little movement at *A*, as this plate moves only through a very small arc even with very great changes in blood pressure. These three movable joints permit the wire *F* to move freely and prevent friction.

DISCUSSION AND CONCLUSIONS

Success in pharmacologic assays depends largely upon the number of comparisons of a given drug on a single animal. The life of the animal may be prolonged by giving smaller doses, and consequently more doses may be given. Since the recorded changes in blood pressure are relatively greater, the accuracy of the assays increases accordingly. The exact change in blood pressure is easily calculated by determining the length of the lever arms and reducing

the result to millimeters of mercury. Furthermore, the apparatus has been used for both pressor and depressor drugs and has proved as satisfactory for one as for the other (Figs. 2 and 3).

In conclusion, it is believed that this manometer has the following advantages over the old type manometer:

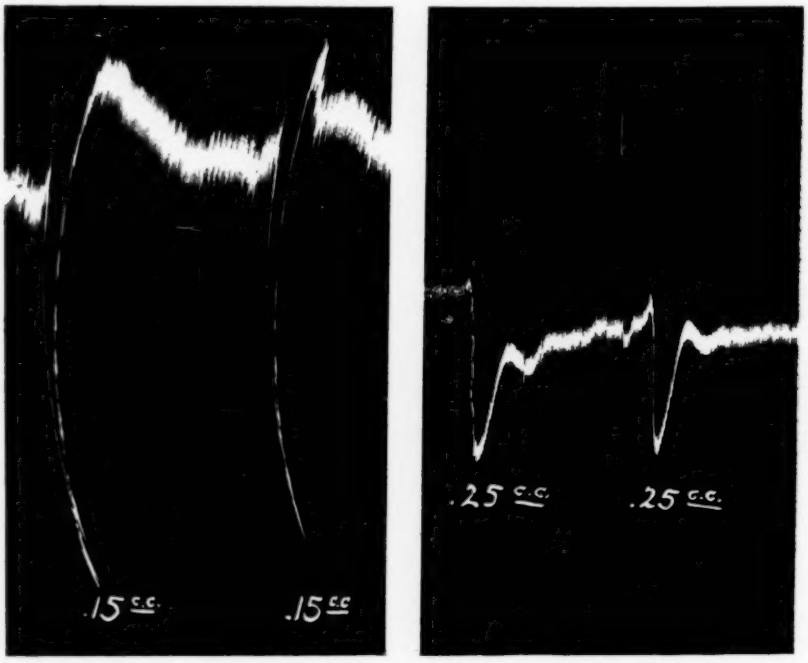

Fig. 3.—Cat, anesthetized with sodium amytal, 65 mg. per kg. Blood pressure recorded from carotid artery. Doses, 0.15 c.c. and 0.25 c.c. of 1-10,000 histamine dihydrochloride given intravenously.

1. Smaller doses of drugs may be given, thus prolonging the life of the animal.

2. Greater changes are produced on the kymographic tracing, thus permitting more accurate interpretation of results.

3. The magnification may be changed by changing the length of the lever arms; thus, very small changes of pressure may be greatly enlarged in the tracing.

A NEW TEST TUBE RACK FOR USE IN SEROLOGY AND BACTERIOLOGY*

By J. Arthur Reyniers, M.S., Notre Dame, Ind.

AT SOME time or another every technician has expressed dissatisfaction with the test tube racks now in use. The average test tube rack is bulky, nonadjustable and consequently will hold only a limited number of tubes of the same diameter or smaller than the diameter for which the rack is designed. Tubes held in larger holes than the size for which they are intended rattle disagreeably and have a tendency to slip from the rack. Furthermore the average rack tends to obscure if not to hide the contents of the test tube. Racks designed up to the present to overcome some of these difficulties are entirely too specialized and ex-

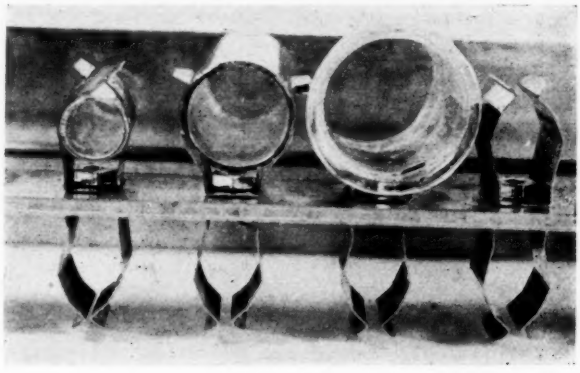

Fig. 1.—Showing the arrangement of the clips on the clip bar and the method of holding test tubes of different diameters.

pensive for the average laboratory. I believe that my rack has none of the disadvantages mentioned and that it has, moreover, decided advantages over any other rack I have seen.

The frame of the rack is constructed of a single piece of heavy brass or stainless steel, highly polished, bent into wings about two inches high. A bar of the same material as the frame is fitted into slots milled into the wings, and a drop of solder fixes the bar in place. Beneath this bar and parallel to it is a rod. Clips are mounted on either side of the bar. These clips are made of phosphor bronze and are designed for extreme flexibility and holding qualities. Experiment with over fifty different clip designs shows that great pressure is not required to hold the tubes firmly if the pressure is applied correctly. From Fig. 3 it can be seen that the test tube is held at six points of contact and not in a band-like grasp. Four of these points of contact press the tube back against the other points to hold it firmly in place. Flexibility is offered by the small indentations

*From the Bacteriology Laboratories, University of Notre Dame.
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at the base of the clip, the manner in which the clip is held to the bar and the length of the clip arms.

The new rack has the following decided advantages over other racks. It is simple and compact in construction and inexpensive to make. It will hold twice the number of tubes in the space the older models required. It will hold firmly and securely any tube between $\frac{1}{4}$ inch to $\frac{3}{4}$ inch in diameter so that the contents

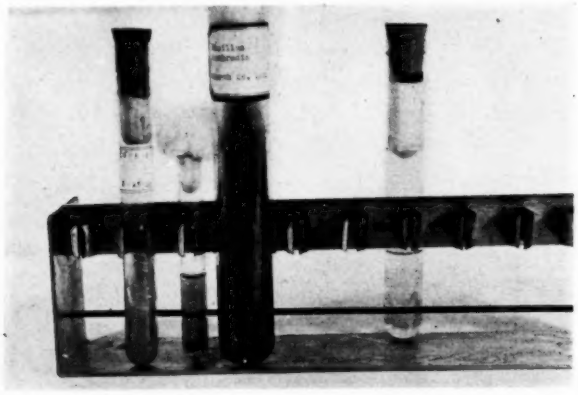

Fig. 2.—Showing the perfect visibility of the tube contents obtained with this method of supporting test tubes.

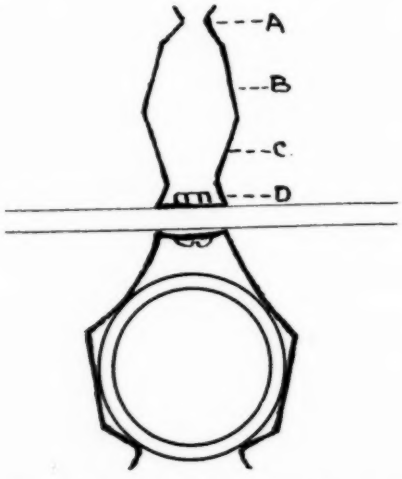

Fig. 3.—Showing the principle on which the expanded and unexpanded clip holds the test tube.

(A) Wing to facilitate the removal of the tube and to act as a point of contact. (B) Second point of contact with the test tube. (C) Point of contact against which the tube is forced by points (A) and (B). (D) Indentations in the base of the clip which with the base allow a maximum of expansion.

of the tube are visible along its entire length—a feature which will be appreciated by all those who have many tests to read. It will hold any shape test tube, will withstand autoclaving, and because of the simple construction of the base is firm and stable. Lastly the rack is "student proof," holding up well under rough usage. The clips as experiment and constant usage in the bacteriologic laboratories of the University of Notre Dame for over a year has shown, do

not spread to any appreciable extent and if strained by a grossly oversize test tube can easily be replaced with a new clip or the old damaged clip may be pinched together by the fingers.

The principle on which these racks are based, namely, the clips, can be used in many different ways. These clips may be adapted to racks for fermentation tubes, experimental museum exhibitions and culture libraries, to mention only a few examples.

The racks are used in the same manner as the older models. Test tubes are pushed into the clips from above and may be drawn out the same way or quicker by simply pulling them out and away from the clip.

A LABORATORY CHRONOGRAPH PROVIDING INTERMITTENT AND CONSTANT CURRENT FROM A DIRECT CURRENT LINE OF 110 VOLTS*

By O. G. Harne, Baltimore, Md.

A SIMPLE electrical chronometer operating upon 110 volts A.C. has recently appeared and was described by Chillingworth.[1] In this chronometer a battery of cells is used to activate the laboratory apparatus. In the present paper we offer a circuit operating upon D.C. lines, and used in our own installation.

Wherever a 110 volt D.C. line is available, the circuit presented in Fig. 1 will provide a convenient laboratory combination of intermittent and direct currents. One or many lines to the laboratory tables may be had offering intermittent current through a "single" cycle circuit breaker serving as a chronometer giving impulses at second intervals. A 1/60 cycle circuit breaker U^3 is propelled by the same motor providing minute intervals. The potential through the breaker mechanism is controlled by P_2, and is read off on V.[1] Direct current is supplied to any number of tables through P_3 in quantities up to 3 amperes with a potential controlled by P_4 at each table, ranging from 0 to 15 volts. (Equivalent to 10 dry cells.) This unit of the combination is independent, and may be used alone as a complete substitute for batteries. It may be used to replace the battery circuit in apparatus already installed, without changing the internal wiring,[2] or altering the commutator discs.[3]

This circuit provides three lines to each laboratory table; (1) intermittent circuit, (2) ground, and (3) the direct current circuit. Line 1 is made up of the two leads from the rotors of the circuit breakers, fed through contact inserts *C*. Line 2 is a common ground for both intermittent and direct current. Line 3 arises from the arm of P_3 and feeds the table potentiometers. The voltage is controlled by P_4 and read off on V_2. Potential changes are quickly made by rotating knob on arm of P_4.

All the apparatus mentioned thus far may be purchased upon the open market at low cost, except the circuit breakers. These are easily prepared. Detail description is given in a previous article.[3] The number of lines through the circuit breakers is limited only by the number of contact inserts *C*. The number of different time intervals possible with any one setting of P_1 is limited only by the number of circuit breakers shunted into the circuit of Line 1. The selection of time intervals depends upon individual needs, but regardless of the intervals selected, the apparatus operates efficiently.

REFERENCES

1. Chillingworth, Felix P.: A New Time Marker, J. Lab. & Clin. Med. 16: 912, 1931.
2. Porter, W. T.: An Electrical Clock, Proc. Am. J. Physiol. 31: 28, 1913.
3. Harne, O. G.: The University of Maryland Chronograph, Its Construction, Advantages, and Application to the Needs of Physiological and Pharmacological Laboratories, J. Lab. & Clin. Med. 11: 641, 1926.

*From the Laboratory of The Department of Physiology, University of Maryland.
Received for publication, September 4, 1931.

Fig. 1.—University of Maryland New Chronograph wiring diagram and parts used. *S*, switch, 110 volts; P_1, 260 ohm potentiometer, capacity 1 ampere; P_2, 42 ohm potentiometer, capacity 3 amperes; P_3, 40 ohm potentiometer, capacity 3 amperes; P_4, 60 ohm potentiometer, capacity 2 amperes; *C*, contacts; V_1, volt meters, 0-15, type P-30, Hoyt; V_2, volt meter, 0-15, type 557, Hoyt; *J*, phone jacks; *U''*, contact unit for minutes; R_2-R_1, rotating arm containing brush. (-----) crosses 3 leads connecting chronograph to table units. For details of *U''* and U^3 see Harne, O. G.: J. Lab. & Clin. Med. **11**: 641, 1926.

AN IMPROVED ETHER BOTTLE FOR ANIMAL ANESTHESIA*

By R. H. K. Foster, B. Chem. Eng., Chicago, Ill.

THE regulator herein described was developed for use with artificial respiration as provided by the C. F. Palmer (London, S. W. 2) artificial-respiration machine. This machine is provided with inlet and outlet valves arranged in such a way that air passes only in one direction through an ether bottle to a Y-tube attached to the trachea of the animal. The animal exhales through the other arm of the Y-tube which connects with the outlet valves in the machine. However the ether regulator can be used with other types of artificial-respiration machines. It can be used in spontaneous respiration if two one-way valves as used in metabolism apparatus are employed in the circuit to prevent rebreathing through the bottle.

Briefly, the advantages of this ether-air regulator are: a permanent record of the anesthesia may be made on the kymograph tracing; the ether vapor concentration is measured and easily adjusted at all times; a constant level of ether is maintained in the bottle thereby permitting uniform evaporation of the liquid; and finally, the supply is replenished without the disadvantage of removing the bottle lid frequently.

The figure illustrates the ether regulator with dimensions given in inches. The fittings are made of brass and soldered to a standard mason jar screw cap. A small tin can with a tightly fitting cork stopper is used as a reservoir for the ether. A hole is punched in the bottom and the can then soldered around this hole to the stopcock *L*. The other end of the tube from *L* runs 4 inches below the mason jar cap. This brings the open end of the tube from the reservoir to about the middle of a one-quart jar. As the liquid evaporates, air bubbles into the tube displacing ether from the reservoir. The stopper in the reservoir must of course be kept tightly inserted except when refilling when the valve *L* is closed.

Air from the artificial-respiration machine enters at *A* and goes to the animal from the exit *B*. Tube *A* is flattened at one end to form a slot one-eighth inch wide by one inch long. Tube *A* is soldered in place over a similar slot in the sleeve *E*. Sleeve *E* has an excursion of one inch on the vertical tube *H*. In the middle of *H* there is soldered a thin partition, *G*. Two slots, each one-eighth inch wide by one inch long are cut in tube *H* as shown at *F*. The sleeve *E* slides over these slots, being kept in position by the guide pin *O* as shown.

When the sleeve with the side arm *A* is in the midposition half the air will pass down into the ether bottle and half will pass upward around the by-pass *D*, the two streams again merging in *B*. By having the upper inch of tube *H* graduated in eighths, a scale is obtained whereby accurate adjustment of the mixture is easily effected. The sleeve is maintained in a fixed position by the heavy spring-bronze friction strip *K*.

*From the Pharmacological Laboratory, University of Chicago.
Received for publication, October 8, 1931.

The lug *J* is for the purpose of fastening a thread to a writing lever for recording on smoked paper. In operating it is desirable to place two styluses to mark base lines in order to indicate the maximum and minimum excursions of the ether-air-mixture recording lever.

The sleeve should fit snugly on *H*, yet slide with ease. The by-pass *D* simply slips over the tops of tubes *H* and *N*. *M* is a flat brass strip to which the various tubes are soldered and which in turn is soldered to the mason jar cap. It also facilitates screwing the cap on the jar. Tube *A* must be three-fourths of an inch in diameter, so that the one end when flattened will have a slot one inch long.

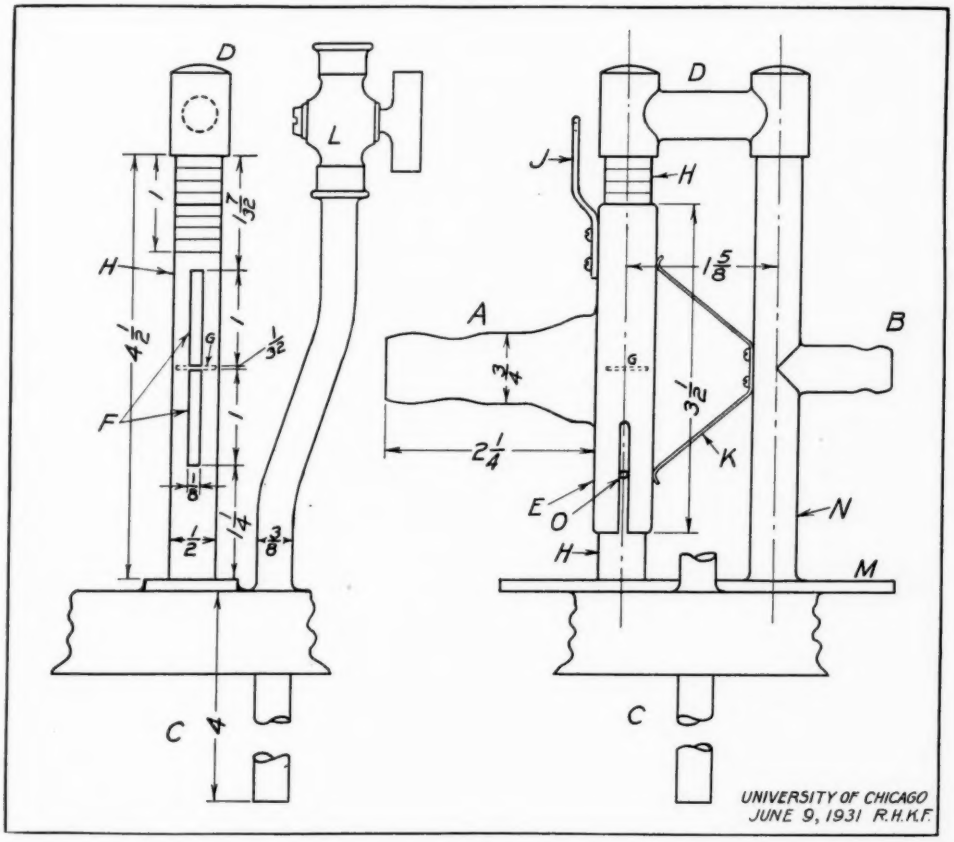

Fig. 1.

The sleeve is of such length that when completely lowered all the air passes through the ether bottle and when completely raised none passes through.

In order that the adjustment of the writing lever will not be disturbed the ether bottle is anchored to a ringstand by clamps.

SUMMARY

1. An ether-vapor regulator is described by means of which a permanent record of the anesthesia is obtained. Changes in circulation or respiration can be correlated with any changes in the ether-vapor concentration.

2. The proportion of ether-vapor to air can easily and fairly accurately be regulated.

3. A constant ether level can be maintained in the bottle, thus eliminating variations of ether-vapor concentration due to changes in liquid level.

4. The ether bottle can be refilled without removing the mason jar cap. Thus in a prolonged experiment or series of experiments the jar can be anchored in place, obviating the necessity of readjusting the recording lever each time the bottle is refilled.

5. The device can be used in studying effects of changes in vapor concentration and is suited to other volatile anesthetics such as chloroform.

6. The device is designed for one-way air passage, thus eliminating rebreathing expired air from the dead space and in addition using less ether.

In conclusion, the author wishes to express thanks to Dr. H. B. Van Dyke and Dr. A. J. Carlson for their cooperation in making suggestions and to Mr. Gus Lutz whose mechanical skill and ingenuity aided materially in producing a satisfactory working mechanism.

METHOD FOR STAINING FECAL PROTOZOA*

By Rawson J. Pickard, M.D., and Clara Rice, R.N., San Diego, Calif.

THE case of a physician who for years had had pathologic fatigue, brief diarrheas, and abdominal distress, and who had examined his own stools many times both in fresh preparations and with iodine, with no other finding than innumerable yeast cells, recently reimpressed on us the necessity for making all examinations for fecal protozoa from wet fixed preparations stained with iron hematoxylin. The innumerable "yeast cells" proved to be equally numerous cysts of the small race of E. histolytica (E. Hartmanni).

We have long used a technic modified from that given by Langeron,[1] by a rapid method, obtaining preparations suited for species diagnosis, and by a slower technic, getting regularly pictures fully as detailed as those obtained by the somewhat lengthy Schaudinn fixation. The examination for proper differentiation in creosote gives a clarity which is not possible with examination in water and the species diagnosis may be made then if permanent slides are not desired. If further differentiation is required the creosote is readily removed with alcohol or if differentiation has gone too far the steps may be retraced further back. Also, due to the difficulty of obtaining the necessary quantities of ethyl alcohol, we found that the ordinary "completely denatured alcohol, Formula 1," is a perfectly satisfactory substitute after it is clarified and purified by distillation over NaOH, giving a water clear product of 95 per cent alcohols.

TECHNIC

1. Fixation in alcoholic Bouin's solution, one hour, room temperature.
2. Harden five minutes, 95 per cent alcohol.
3. Seventy per cent alcohol, five minutes, may leave in this indefinitely.
4. Wash in two changes water (tap).
5. Ferric alum 3 per cent 15 minutes at 32° C., rapid technic (12 hours room temperature for slow technic for precision of detail).
6. Wash, two changes water (distilled).
7. One per cent hematoxylin, 15 minutes at 32° C., (12 hours room temperature, slow technic).
8. Wash in water.
9. Differentiate in 3 per cent ferric alum at 32° C., (at room temperature, slow technic).
10. Wash in water.
11. Wash in 95 per cent alcohol.
12. Clear in creosote, drain off excess, examine with 50X oil immersion using a cover glass to protect the lens from creosote. If permanent preparation is

*Received for publication, September 17, 1931.

desired and the differentiation is correct for the protozoa found present, remove the cover.

13. Clear in xylol, mount in xylol balsam.

Age the 1 per cent hematoxylin six weeks in sunlight, add a crystal of thymol to preserve. Alcoholic Bouin's solution made fresh daily from: 30 c.c. of 80 per cent alcohol saturated with picric acid, 12 c.c. formalin (40 per cent), and 3 c.c. glacial acetic acid. Coplin jars for various solutions. For the fixation the flat Laveran "boxes" are preferable. Differentiation varies from a few seconds for flagellates to several minutes for the larger amebic cysts; it must be controlled under the microscope.

REFERENCE

1. Langeron, M.: Précis de Microscopie, ed. 4, Paris, 1925, Masson.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

B. DIPHTHERIAE: Stain for, Gutstein, M. and Neisser, H. Centralbl. Bakteriol. **108:** 253, 1928.

1. Stain two to three minutes with 1 per cent aqueous Azure II.
2. Wash in water.
3. Differentiate with 3 per cent acetic acid (one to three seconds).

Granules are reddish-*violet;* bodies blue.

or

1. Stain two to three minutes with 1 per cent aqueous Azure II.
2. Wash with water.
3. Wash with aqueous chrysalinine.

Granules are black; body yellow.

ALLERGIC ASTHMA: Nonspecific Desensitization Therapy in: The Eosinophilic Index as a Guide to Intramuscular Injection of Venom Protein, Spangler, R. H. Arch. Int. Med. **36:** 779, 1925.

It would be of value if a method could be established for regulating the strength of the dose and the frequency with which a protein injection should be given in order to keep a patient nonspecifically desensitized.

Spangler, who has used intramuscular injections of venom protein (crotalin) for many years, suggests a study of the eosinophiles in the differential count (eosinophilic index) as a satisfactory guide.

As a rule, the highest rise in eosinophiles after crotalin injections (1/400 to 1/50 grain) occurs by the second or third day.

It is his practice not to repeat an injection if the eosinophile count has not dropped to normal or to the preinjection level by the fifth day after injection. Moreover, it is wise not to increase the dose if any given dose is producing an eosinophilia of 8 to 10 per cent by the second or third day after injection.

KOTTMAN REACTION: Diagnostic Value of the Kottman Reaction in Thyroid Dysfunction. Am. Jour. Med. Sc. **172:** 84, 1926.

From a study of 101 cases, including hyperthyroidism and various pathologic conditions, Katayama concludes that:

It is generally conceded among physiologists and clinicians that the determination of the basal metabolic rate is the most dependable laboratory index of thyroid activity. Increased thyroid secretion produces a lowering of the tolerance for carbohydrate, but there are numerous other conditions in which the tolerance for carbohydrate may be diminished. Hence the occurrence of high blood and urine sugar curve after the ingestion of glucose is not in itself indicative of hyperthyroidism. In hyperthyroidism, however, the blood and urine sugar curves after glucose furnish information concerning a phase of carbohydrate metabolism which is not gauged by the basal metabolic rate.

The basis of the Kottmann reaction is obscure, and hence it is difficult to say in what manner thyroid activity influences it.

BRAIN TUMOR: Yellow Spinal Fluid Associated with Tumor of the Brain, Comfort, M. W. Arch. Neurol. and Psychiat. **15**: 751, 1926.

The present review has led to the belief that a definite localizing and prognostic significance is attached to the presence of xanthochromic fluids in cases of brain tumor, and that the following conclusions, although based on the study of a comparatively small group of cases, are justified.

Yellow spinal fluid associated with tumor of the brain occurs much more frequently than the reported cases indicate. In the present series, xanthochromia occurred in approximately 20 per cent of the cases of tumor of the brain.

Xanthochromic spinal fluid associated with a tumor of the brain indicates the involvement of the ventricle or external surface of the brain, and that the tumor is sufficiently vascular and soft, or so surrounded by engorged vessels, as to permit hemorrhage or transudation into the cerebrospinal fluid.

The available evidence points to a hemolytic origin for the xanthochromia accompanying tumor of the brain; a serous origin, while theoretically possible, has not been proved.

The hemorrhages accounting for the coloration are conceived of as being scanty and repeated.

The number of erythrocytes present is a measure of the softness and the vascularity of the tumor.

Xanthochromia combined with the presence of many erythrocytes, indicating the presence of a soft vascular tumor prone to hemorrhage, should greatly increase the risk of, and possibly contraindicate, those operative procedures which markedly reduce the intraventricular pressure.

DIABETES: Relation of Abdominal and Rectal Infections to the Pathogenesis of Diabetes Mellitus, Visher, J. W. Am. Jour. Med. Sc. 171: 836, 1926.

The underlying pathologic change in diabetes in many cases is a pancreatitis.

This pancreatitis may originate in acute infectious diseases, and from hematogenous focal infections.

Five cases of diabetes mellitus apparently secondary to abdominal and rectal infections are reported, with improvement following surgical intervention.

The opinion is ventured that in these cases the infection reached the pancreas through the lymphatics, either directly or by way of the portal circulation.

The conclusion is suggested that abdominal and rectal infections are important etiologic factors in the etiology of diabetes mellitus.

MERCUROCHROME-220 SOLUBLE: The Use of, in the Treatment of Infectious Diseases of the Skin, Young, H. H., Hill, J. H., and Denny, W. L. Arch. Dermat. and Syph. **13**: 465, 1926.

The following cases in which mercurochrome was used intravenously are reported:

Twenty-four patients with erysipelas, of whom twenty (83.3 per cent) were cured or greatly improved; eleven patients with furuncles and carbuncles, of whom ten were cured and one greatly improved; four cases of chancroidal ulcerations, all of which healed rapidly; thirty-six patients with cellulitis and abscesses, of whom twenty-one (58.3 per cent) recovered promptly with no other treatment, and nine of whom (25 per cent) showed marked improvement and recovery after mercurochrome therapy, other treatment being also given, making a total of thirty cases, or 83.3 per cent, cured or greatly improved; two patients with gas gangrene, in both of whom the infection was eliminated; one patient with diabetic gangrene, in whom an accompanying infection was checked; three patients with pemphigus, all of whom showed marked improvement, two of them so far without relapse, one with relapse; four patients with psoriasis, two of whom are apparently cured, one markedly improved, and one improved, but with a relapse; one patient with eczema, temporarily improved; one patient with syphilis with exterior skin lesions, in whom the lesions were healed;

forty-four patients with leprosy, twenty-eight (63.5 per cent) of whom were improved and with remarkable disappearance of the skin ulcers.

HYPERGLYCEMIA: The Relative Blood Volumes in Diabetes Mellitus, Foshay, L. Arch. Int. Med. **36**: 889, 1925.

Polyuria and exsiccation occur in diabetes in proportion to the severity of the disease. It seems probable, then, that diuresis and renal permeability have some influence in determining the course. If so, diabetes in the young should show more evidence of blood and tissue dehydration than in the elderly in whom the disease is less apt to be as severe.

The studies of Foshay were designed to secure evidence as to the correctness of these suppositions.

Tissue exsiccation was determined by clinical observations of the skin, subcutaneous tissues, and mucus membranes; anhydremia and hydremia by the relative volumes of serum calculated by the method of Stewart.

Studies were made of 13 young diabetics; 15 arteriosclerotic diabetics presenting acute exacerbations, and 8 patients who had been long under treatment, as a result of which the following conclusions are presented:

Diabetes mellitus produces marked changes in water distribution and in the total water balance of the body. The degree of these changes is modified by the degree of hyperglycemia and the rate of onset; and their character by vascular disease and renal permeability.

Reduction of hyperglycemia or the action of insulin may produce either a decrease or increase in the concentration and viscosity of the blood depending, in either case, on the antecedent water content.

GONORRHEA: The Diagnosis of, Through Intracutaneous Vaccine Injections, Kohler, H. Ztschr. f. urol. Chir. **19**: 54, 1926.

As a result of his experimental studies Kohler concludes that the induration of the skin produced by the intracutaneous injection of 0.5 c.c. of gonococcus vaccine containing 3,000 to 10,000 organisms per cubic centimeter, if it persists more than twenty-four hours, is specific for gonorrheal infection, either present or recent.

BLOOD: Physical and Chemical Studies of Human, from Cases of Diabetes Mellitus, Foshay, L. Arch. Int. Med. **37**: 18, 1926.

The following studies were made on defibrinated blood:

1. Erythrocyte count: in duplicate and checking to 250,000 per cu. mm.

2. Electrical conductivity of whole blood and serum, the result expressed as specific conductivities ($K \times 10^4$ at 50° C.).

3. Relative volumes of serum and erythrocytes by calculation.

4. Average erythrocyte volume: the relative volume of erythrocytes in cu. mm. divided by the number of erythrocytes per cu. mm. Expressed as cubic microns.

5. Glucose: in serum and whole blood determined by Folin-Wu technic.

6. Chlorine: determined by Whitehorn's method (Jour. Biol. Chem., February, 1921, xlv, 449).

7. Corpuscular chlorine and corpuscular glucose concentrations:

$$\%\text{ in cells} = \frac{\%\text{ in whole blood} - (\text{serum volume }\% \times \%\text{ in serum})}{\text{cell volume }\%.}$$

8. Grams glucose and grams chlorine per erythrocyte:

$$\frac{\text{Grams substance per 1 cu. mm. cells} \times \text{cell volume }\%}{\text{Number of cells per cu. mm.}} = \text{Grams substance per cell.}$$

Summary: The conductivity of whole blood and serum varies inversely as the concentration:

In diabetes a "conductivity chloride discrepancy" occurs, the cause of which is not known.

The normal erythrocyte volume is approximately 76 cubic microns.

In all the young diabetic patients and in two of the arteriosclerotic patients with acute exacerbations, the volume average 85 to 90 cubic microns. With recovery the volume returned to normal.

The order of events would seem to be as follows: In young patients hyperglycemia produces a dehydration of the blood and of the fixed tissues, thus causing a nonvolatile acidosis with consequent increase in erythrocyte volume by reason of water transference. This may be considered the predisposing cause of diabetic acidosis. As cellular metabolism becomes more and more abnormal there is a production of ketone acids in increasingly larger amounts; this further diminishes the plasma alkaline reserve. In cases of severe acidosis with rapid onset, no doubt both processes become important factors.

In elderly, arteriosclerotic patients, hyperglycemia does not result in dehydration and acidosis; hence no water transference from the plasma to the erythrocytes and no increase in erythrocyte volume occur. The patients live in relative comfort and are not in constant danger of acidosis and coma. If a serious dietary indiscretion or acute infection supervenes, then dehydration, acidosis, and coma occur just as in the young diabetic patient.

In general, the corpuscular glucose is usually a little less than the concentration in the serum; however, with increasing hyperglycemia the greater portion of the glucose is found in the serum and conversely.

Concerning the distribution of chlorine, the only constant finding in diabetes is that in the presence of hyperglycemia the chlorine concentration of whole blood is always diminished. A consistent relationship between hyperglycemia and corpuscular chlorine was not found.

In untreated, arteriosclerotic diabetic patients there was no evidence of diminished blood alkalinity unless the patient was in coma.

WASSERMANN REACTION: The Rational Use, Haythorn, S. R. Ann. Clin. Med. 4: 493, 1925.

Haythorn uses the laboratory requisition below in order to acquire reliable statistics:

WASSERMANN REQUISITION

Please fill out for research data. The laboratory reserves the right to withhold the report until the requisition blank has been filled out.

Blood

Name _____ Spinal Fluid Date_____ Hour_____
Hospital Address _____ Physician_____
Nationality _____ Sex_____ Married_____ Age_____
Chief Complaint _____
Clinical Diagnosis _____
Is the patient suffering from any febrile disease?_____
Clinical indications of specific disease (place circle around type indicated)
None, Questionable, Primary, Secondary, Tertiary, Hereditary, Cerebrospinal.
Duration of suspected specific disease_____
Results of previous Wassermann tests_____
Approximate number of arsphenamine treatments_____
How long since last treatment?_____
Other antiluetic treatments _____
If your reason for requesting the test has not appeared in filling out the above blank, will you kindly state it here_____
_____
Pregnant_____ Month_____ Miscarriages_____
Postpartum_____ Days_____

TUBERCULOSIS AND ASPERGILLOSIS: Aspergillosis of the Lungs and Its Association with Tuberculosis, Lapham, M. E. Jour. Am. Med. Assn. 87: 1032, 1926.

Lapham summarizes from the literature the reports of pulmonary aspergillosis in human beings; calls attention to the difficulty of differentiating this condition from tuberculosis and

expresses the belief that it is a more frequent complication or concomitant of tuberculosis than is recognized. She argues for routine cultures of the sputum in tuberculosis and says:

"Here is a disease that is capable of causing pleurisy, acute and chronic; bronchitis, acute and chronic; pneumonias, acute and chronic; emphysema; bronchiectasis; atelectasis; sclerotic fibrosis; tubercles; cavities; endarteritis; thrombosis; infarcts; hemorrhages; asthma. Would it be strange if such a disease should seriously complicate or even inhibit recovery in a case of tuberculosis?"

She concludes that we are thoroughly ignorant of the frequency of aspergillosis both as a primary and as a secondary disease.

We have no idea how much aspergillosis of the lungs predisposes to tuberculosis in human beings or in cows.

We do not know how much it impedes or even inhibits recovery in cases of tuberculosis.

We have no idea whatever as to its association with acute respiratory diseases.

We know that it affects cows much as it does human beings.

We know that it gives the same reaction to tuberculin that tuberculosis does.

Should we apply this knowledge to the study of the tuberculosis of dairy herds?

In order to gain adequate information as to the frequency and importance of this disease, should not a systematic research study be made:

1. By determining the percentage of aspergillosis cases among the tuberculosis cases in the large tuberculosis sanatoriums.

2. By determining the percentage of cases of aspergillosis in the lungs at necropsies.

3. By determining the percentage of cases of aspergillosis in cases of respiratory diseases.

4. By applying these prnicipies to dairy herds.

FOREIGN BODIES IN THE LUNG: Pathologic Changes in Lung Tissue as the Result of Foreign Bodies of Long Sojourn, Manges, W. F. Jour. Am. Med. Assn. **87**: 987, 1926.

For the purpose of this paper, a foreign body is one of long sojourn after it has been present in the air passages for two months or more and up to as many as thirty-five years, except that in one or two instances the sojourn has been less than two months but the pathologic changes are unusual. Serious pathologic changes do occur at times in cases of much shorter duration, but these are more or less constantly the acute type, such as infection, emphysema or atelectasis, with which physicians are quite familiar.

An aspirated foreign body in any portion of the tracheobronchial tree will sooner or later cause extensive permanent injury.

There is great variation as to the length of time a foreign body may be present before causing extensive changes, but those that interfere with drainage do, as a rule, cause injury early.

The permanent pathologic change is distal to the point at which drainage is blocked. The foreign body is at or distal to this point.

The end-results are in the nature of atelectasis, fibrosis, bronchiectasis and chronic abscess, with varying quantities of purulent exudate. Hemorrhage is common; tuberculosis is often suspected but is rarely present. The other lung remains remarkably free from pathologic change.

Manges believes that many of the one-sided, chronic, basal infections are the result of foreign body, regardless of history or of roentgenographic shadow of foreign body. Such lesions should at least be investigated bronchoscopically, and many should be treated in this manner.

FOREIGN BODIES IN AIR PASSAGES: Live Fishes Impacted in Food and Air Passages of Man, Gudger, E. W. Arch. Path. and Lab. Med. **2**: 355, 1926.

Gudger, who is bibliographer to the American Museum of Natural History, has collected from the literature all the reports of impaction of live fishes in the human throat and air-passages.

The paper is most readable and of extreme interest but does not lend itself to abstraction.

CARCINOMA: Grading the Malignancy of Carcinoma, Grading and Practical Application, Broders, A. C. Arch. Path. and Lab. Med. 2: 376, 1926.

Broders uses the following classification:

If an epithelioma shows a marked tendency to differentiate, that is, if about three-fourths of its structure is differentiated epithelium and one-fourth undifferentiated, it is graded 1; if the differentiated and undifferentiated epithelium are about equal, it is graded 2; if the undifferentiated epithelium forms about three-fourths and the differentiated about one-fourth of the growth, it is graded 3; if there is no tendency of the cells to differentiate, it is graded 4. Of course, the number of mitotic figures and the number of cells with single large deeply staining nucleoli (one-eyed cells) play an important part in the grading.

He further modified this conception as follows:

Instead of a Grade 1 epithelioma in which about three-fourths of the cells are differentiated and one-fourth undifferentiated, should be substituted a Grade 1 epithelioma, one in which differentiation or self-control ranges from almost 100 to 75 per cent, and undifferentiation from almost nothing to 25 per cent; a Grade 2 epithelioma, one in which differentiation or self-control ranges from 75 to 50 per cent, and undifferentiation from 25 to 50 per cent; a Grade 3 epithelioma one in which differentiation or self-control ranges from 50 to 25 per cent, and undifferentiation from 50 to 75 per cent, and a Grade 4 epithelioma, one in which differentiation or self-control ranges from 25 per cent to practically nothing, and undifferentiation from 75 to practically 100 per cent. So far as an estimation of the ultimate result is concerned, this revision will have no effect on Grades 1 and 2, but will affect slightly Grades 3 and 4, because a small percentage of neoplasms, formerly classified Grade 3, will now be classified Grade 4. In other words, the most malignant of the Grade 3 neoplasms will be classified in Grade 4.

He calls attention to the practical application of this procedure in these words:

"Turning to the practical side of the grading of cancer, it is well known that cancer of Grade 1 shows practically no tendency to metastasize, and therefore in dealing with such neoplasms it does not seem necessary to remove the regional lymph nodes. This saves the patient unnecessary operative procedures. As practically all cancers of Grade 4 with metastasis prove fatal sooner or later, the patients should not be subjected to an operation involving the regional lymph nodes unless they are in close proximity to the primary growth, cancer for the stomach for example. Judd, New, and Figi believe it is useless to perform block dissection of the neck in the presence of a Grade 4 epithelioma of the lip, tongue, cheeks, floor of the mouth or antrum, etc. In cases of cancer of Grade 2 and in a certain proportion of those of Grade 3, with metastasis, removal of the regional lymph nodes offers a permanent cure in a fair number of cases, as evidenced by the fact that ten (33.3 per cent) of thirty patients with squamous cell epithelioma of the lip of Grades 2 and 3, with metastasis in one group of submaxillary lymph nodes, were living and well on an average of six and one-fifth years after removal of the nodes.

OZENA, BACTERIOLOGY: Bacillus Ozena Foetidae, Perez, and Bacillus Proteus in Ozena, Michailoff, A. Bull. Johns Hopkins Hosp. 39: 158, 1926.

Ozena is a chronic disease of the nose characterized by a mucopurulent discharge, crusts, a specific fetid odor, and atrophy of the turbinate bones.

A bacteriologic study of twenty-eight cases. The bacillus described by Perez was isolated in only seven cases. A bacillus of the proteus group was also found with sufficient frequency to warrent study of its connection with the disease.

Bacteriologic and immunologic studies are reported in detail and the author's conclusions are as follows:

Although the cultural and fermentative properties are insufficient to identify Perez bacillus as atypical Proteus, we are justified in thinking that it constitutes a sub-group which

has lost some of its fermentative properties, a frequent occurrence with the most typical proteus, and that B. ozawnae liquefaciens Shiga is a typical B. proteus vulgaris.

The agglutination and agglutinin absorption tests show Perez bacillus to be very closely related to B. proteus; Perez X is intermediate between the two species, having agglutinative properties as strong for Perez bacillus as for B. proteus. Each bacillus of this group has a serologic individuality more or less close to the Perez or proteus species. Of the fourteen bacilli, no two are identical. Agglutinin absorption shows clearly that the motile strains, B. ozaenae liquefaciens Shiga, and B. proteus vulgaris, as a rule, have their coagglutinative branches reduced or removed when absorption is done with the motile bacillus, and vice versa; they are more closely related to each other than to the nonmotile Perez species and can be identified as belonging to the same group as B. proteus.

Complement fixation shows that the two varieties, motile and nonmotile, have a very close relation. Some Perez and Proteus bacilli have identical properties as antigens against a given serum and this reaction appears to show closer relationship than the agglutination or agglutinin absorption test. There is no correlation between the complement-fixation and agglutination test.

The more specific flocculation reaction also shows the organisms to be very closely related, but only Perez II and Hofer's Perez bacilli have identical properties, while of these only the latter shows flocculation with B. proteus.

B. proteus, B. perez, and B. ozaenae liquefaciens have identical pathologic properties. Each produces the four different types of infection: (a) toxico-septicemic congestive and hemorrhagic lesions; (b) chronic pyemic, mucosal, and endothelial exudative and proliferative lesions; (c) local exudative lesions; and (d) special necrotic lesions due to complex pathologic processes.

All the strains studied produce a toxin identical with that of Hofer's Perez bacillus and Proteus vulgaris.

Cross immunization shows that Perez bacillus and B. proteus have identical immunizing properties, protecting completely or producing sufficient protection to indicate their identity or very close relationship.

On the basis of the above conclusions it follows that the Perez bacillus is a member of the large group of Proteus bacilli.

The etiologic relation of the Proteus-Perez group to ozaenae fetida has been suggested by many workers because of the frequent finding of these organisms in the nasal discharge of such cases. The Proteus group exhibits an affinity for the blood vessels and mucosae, has pathogenic power, producing chronic necrotic lesions and nasal ulceration and discharge. We never produced green crusts or atrophy of the turbinate bones in the rabbit, and therefore we cannot conclude that in human beings B. proteus produces ozaena fetida, and that this bacillus acts as a primary etiologic agent. The frequency of its occurrence and the specific malodor found in the cultures show that B. proteus in involved in the pathogenesis of ozaena, and whether implanted primarily or secondarily, is the cause of the fetor, discharge, and ulceration.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Histopathology of Skin Diseases*

IT IS, of course, fortunate for the patient that few die as a result of diseases of the skin per se, but unfortunate from a scientific standpoint in that this fact has undoubtedly retarded advancement in the understanding of these conditions.

There are volumes galore upon the symptomatology of diseases of the skin and innumerable compendiums of formulae for their treatment. But there are few volumes, indeed, which present in an authoritative and comprehensive way the details of the histopathology of diseases of the skin.

Dr. McCarthy and the publishers are to be congratulated, and the thanks of the medical profession are due to Mr. Truxton Beale through whose generosity the publication was made possible, for the eminently successful completion of this publication.

Without doubt, this book will become an authoritative reference for the profession at large and will establish a standard for similar works of the future.

Dr. McCarthy, in his presentation of the subject shows not only a thorough comprehension of its many ramifications, evidently based upon an extensive and practical experience, but also a thorough knowledge of the literature.

As is well known, a well conceived and well executed illustration is often more informative than pages of descriptive text. This book, therefore, is well and profusely illustrated many of the plates being in color. While microphotographs are not neglected, great reliance has wisely been placed upon careful, accurate, and excellent drawings in which the essential features can be clearly shown and the unessential minimized.

The reproduction of the drawings and especially of the colored plates are exceptionally well done and when, as in this book, both text and illustrations are equally well done and truly complementary, the result is a volume of the greatest value to students of the subject.

This book may be recommended without reserve as a most valuable and useful contribution to an important and, in many ways, obscure subject.

The Regulation of Size As Illustrated In Unicellular Organisms†

SIZE is one of the chief properties of living organisms, and the problem of size regulation is one to which the attention of science has long been turned and in connection with which a number of more or less directly related observations have been recorded.

In this book the pertinent data in this field have been collected, arranged, and weighed by Professor Adolph so that the investigations of many observers are here outlined as a coordinated whole.

The book covers a field as yet but little tilled and constitutes a contribution of distinct interest to biology and kindred sciences.

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*Histopathology of Skin Diseases. By Lee McCarthy, M.D., Associate Clinical Professor of Dermatology, Georgetown University Medical School, etc. Cloth, 513 pages, 251 illustrations, many in color. The C. V. Mosby Co., St. Louis, Mo.

†The Regulation of Size as Illustrated in Unicellular Organisms. By Edward A. Adolph, Associate Professor of Physiology in the University of Rochester. Cloth, 230 pages, 66 figures. C. C. Thomas, Springfield, Ill.

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EDITORIAL

Protein Digestion and Food Allergy

SINCE men first reached that stage where they could compare experiences, food idiosyncrasy has probably been a recognized entity. The interpretation of these isolated individualistic manifestations has necessarily varied at different epochs, dependent upon the medical teachings of the day, some of which indeed have persisted and still color our dietary prescriptions. Such for example is probably the popular belief in poisonous combinations of otherwise harmless foods, as sea foods and cream eaten at the same meal. Many of the arbitrary diets for gall bladder disease and colitis still to be found in modern textbooks would appear to be based upon cumulative past experience with cases of food idiosyncrasy.

The striking picture of respiratory anaphylactic death in the guinea pig led to the suggestion that bronchial asthma might be associated with protein sensitization. Knowledge that experimental anaphylaxis may be produced with food proteins resulted in the demonstration that alimentary allergens as well as inhalant allergens could produce allergic manifestations. But at the beginning the

only manifestations which were universally accepted as indubitably allergic were bronchial asthma, vasomotor rhinitis and urticaria. After it had been demonstrated that the allergic response consists primarily in smooth muscle spasm and serous exudation; and that although the reaction is predominantly respiratory in guinea pigs, it is predominantly circulatory and hepatic in rabbits, and gastrointestinal in dogs, horses, hogs and cattle, the conception gradually gained acceptance that a great variety of organs and tissues might be involved in the anaphylactic response and may therefore become responsible for symptoms.

Even in the guinea pig, although the startling picture manifests itself in the respiratory system, other tissues take part in the response. The experiments with sensitized guinea pig uterus are well known. Kendall and Shumate[1] have recently demonstrated and studied quantitatively the anaphylactic reaction in the intestinal musculature of guinea pigs. They found greatest anaphylactic response in the lowermost portions of the small intestine, gradually diminishing upwards, with least response in the lower portion of the duodenum. In the upper duodenum anaphylactic sensitivity was again increased. The participation of the intestinal tract in the allergic response appears to have been experimentally demonstrated. Indeed Templeton and Bollens[2] have devised a method for the study of anaphylaxis, comparable to the uterine strip method, in which they measure increased tonus within the rectum and lower colon as evidence of anaphylactic response.

Studies of gastrointestinal absorption, in the production of experimental anaphylaxis, have necessitated a thorough revision of our understanding of the physiology of digestion. No longer can we state that proteins are completely broken down into their constituent amino acids in the intestines, and absorbed through the mucosa as such.

Assuming that sensitization may occur through intestinal absorption, how does it happen? Schloss and Worthen[3] found by precipitin and anaphylactic tests applied to the urine, that the intestinal tract of normal infants is impermeable to undigested foreign protein. In the presence of gastrointestinal disturbances however, protein was found to be absorbed, either undigested or partially digested and excreted in the urine. This seemed a simple explanation. We become sensitized to food protein as a result of gastrointestinal disturbances which allow the passage of undigested allergen into the blood.

Hettwer and Kriz[4] substantiated these findings by sensitizing guinea pigs to horse serum following its introduction into a temporarily ligated loop of small intestine. They found that stasis and increased intraintestinal pressure were necessary to promote absorption. They obtained similar results following chemical irritation without stasis, by the introduction of horse serum into the unligated intestine together with small amounts of sodium fluoride. Not only did they sensitize guinea pigs in this manner but using the same method they were also able to produce anaphylactic reaction in previously sensitized pigs.

The liver appears to exert a regulatory and detoxicating function during digestion. Presumably it removes undigested protein from the portal blood thereby interfering with its entrance into the systemic circulation. Egg white injected into the circulation appears in the urine sooner when introduced through

an ear vein than through a mesenteric vessel. When injected into a mesenteric vessel, it appears to be removed in part at least by the liver and make its appearance in the bile. In guinea pigs previously sensitized against egg white this protein causes death more rapidly and in small doses when injected directly into the systemic circulation than when introduced into the portal system.

Had investigations stopped at this point the current teachings of physiology would have required no alteration. Proteins are digested in the lumen of the gut and absorbed as amino acids except in the presence of local gastrointestinal disturbance, with resulting increased permeability, when they may be absorbed incompletely digested, and produce sensitization.

But Rosenau[5] succeeded in sensitizing guinea pigs following the *oral* administration of horse serum as did also Hettwer and Kriz. LaRoche, Richet and Saint Girons[6] succeeded in sensitizing guinea pigs to egg white by the oral route. Stokvis[7] as well as Van Alstyne[8] showed that raw egg white taken into the alimentary tract may enter the circulation and be excreted through the urine. Finally Walzer[9] and his collaborators demonstrated by the method of passive transfer that undigested proteins are absorbed and appear in the circulation of normal nonallergic individuals. A small amount of serum from an individual sensitive to egg was introduced into the skin of a nonallergic person. When subsequently the latter ate eggs, a local positive allergic reaction appeared at the site of the intradermal inoculation. The egg in the food was absorbed and carried through the blood to the site of the inoculation, its chemical makeup still sufficiently characteristic of egg white to give a specific response. This was demonstrated repeatedly not only with egg protein but also with fish protein.

Coca[10] has shown that contrary to former belief, protein does pass in minute amounts through dialyzing membranes. If we consider the alimentary tract such a membrane, he has shown that amounts of food protein sufficient to be of clinical significance may thus pass normally into the circulation. This observation explains those of Walzer and his collaborators.

So we must modify our concept to recognize that unaltered protein or only partially digested protein may normally be absorbed into the circulation. This does not clarify the question as to why some persons become sensitized while others do not, but it removes the site of initiation of sensitization from the intestinal mucosa to the tissues themselves. The localization of the allergic response in different organs or tissues remains unexplained.

REFERENCES

1. Kendall, Arthur I., and Shumate, Fredericke O.: The Quantitative Response of Intestine from Sensitized Guinea Pig, to Homologous Protein and to Histamine, J. Infect. Dis. **47**: 267, 1930.
2. Templeton, R. D., and Bollens, W. F.: Attempts to Secure Objective Methods of Studying Mild Anaphylaxis, J. Lab. & Clin. Med. **15**: 585, 1930.
3. Schloss, O. M., and Worthen, T. W.: The Permeability of the Gastrointestinal Tract of Infants to Undigested Protein, Am. J. Dis. Child. **11**: 342, 1926.
4. Hettwer, J. P., and Kriz, R. A.: Absorption of Undigested Protein from the Alimentary Tract as Determined by the Direct Anaphylaxis Test, Am. J. Physiol. **73**: 539, 1925.
5. Rosenau, M. J., and Anderson, J. F.: Further Studies upon the Phenomenon of Anaphylaxis, J. Med. Research **21**: 1, 1909.
6. LaRoche, Guy, Richet, Jr., Charles, and Saint Girons, F. R.: Alimentary Anaphylaxis to Egg. An Experimental Study, Arch. de Med. Exper. et Anat. Path. **26**: 51, 1914. Richet, Jr. Charles: Food Anaphylaxis, J. Allergy **2**: 76, 1931.

7. Stokvis, B. J.: Hühner—eiweiss und Serum—eiweiss und ihr Verhalten zum thierischen Organismus, Zentralbl. f. med. Wiss. **2**: 596, 1864.
8. Van Alstyne, Eleanor V.: The Absorption of Protein without Digestion, Arch. Int. Med. **12**: 372, 1913.
9. Brunner, Matthew, and Walzer, Matthew: Absorption of Undigested Proteins in Human Beings. The Absorption of Unaltered Fish Proteins in Adults, Arch. Int. Med. **44**: 172, 1928.
10. Coca, A. F.: On the Dialyzability of Proteins, J. Immunol. **19**: 405, 1930.

—*W. T. V.*